Thesis for the Degree of Doctor of Medicine.

Vitamin E.

With special Reference to its Application in Clinical Medicine and the Experimental Evidence on which this is founded.

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THE HISTORY OF VITAMIN E.

The discovery of a specific factor in the diet of rats necessary for their normal reproduction was anticipated as early as 1919, when it was found that when these animals were fed on diets of purified foods with vitamins A.B.C. and D. they exhibited normal growth and vigour, but failed to reproduce and showed evidence of gonadal injury. (Osborne, Mendel 1919) Mattill, Conklin 1920).

However, it was not till 1922 that attempts were made to discover the cause for this phenomenon. Evans and Bishop were the first to publish their results and they found that female rats fed on fairly normal diets which contained all the vitamins known at that time failed to reproduce. Oestrus continued and mating was accomplished normally, but pregnancy terminated in foetal death with resorption or abortion as the final stage. It was also found that permanent damage was done to the reproductive tissues of males fed on a similar diet. This anti-sterility factor was termed X. They also found that the addition of whole wheat cereal, fresh lettuce leaves or dried alfalfa grass permitted normal pregnancy. (Evans, Bishop 1922.) Similar results were arrived at at other laboratories. (Mattill 1922.) (Mattill, Stone 1923) (Sure 1924) It was Sure who first termed this factor Vitamin E. (Sure 1924)

After this preliminary research it seemed definitely established that in Rats a factor in their diet was needed to prevent sterility and that this could not be synthesised in the rat's body, so that it came under the definition of a vitamin.

The first studies on its chemical nature were done by Evans and Burr in 1927 but it was not isolated as a crystalline substance till 1936. (Evans, Emerson, Emerson, 1936) and it was two years later that its chemical formula was finally decided upon and that it was synthesised. (Karrer, Fritzche, Ringer, Salomon, 1938) Further details of the discovery of the Chemistry of Vitamin E. will be given in the next section.

It was first used in Clinical Medicine on cases of Habitual Abortion by Vogt-Muller in 1931, but it was not generally used as a therapeutic agent till about 1936 when it began to be used not only in cases of Habitual Abortion but in many other Obstetrical and Gynaecological diseases.

The effect of Vitamin E. deficiency on the nervous and muscular systems of experimental animals was first noted by Evans and Burr in 1928. It was not till after the Pathology of these conditions and their similarity to certain diseases in Clinical Medicine was pointed out in detail by Emerson and Ringsted in 1938, that Vitamin E. came to be used in the treatment of

these conditions. The reports by Bicknall and by Wechsler which appeared in 1940 were among the first on the clinical use of Vitamin E. in nervous diseases. After that its effect was tried in the treatment of many of these, which up to that time had been considered incurable, although often they bore no resemblance to the conditions produced experimentally in animals.

THE CHEMISTRY OF VITAMIN E.

Vitamin E. was found to be present in greatest quantities in the unsaponifiable fraction of wheat germ oil and is also present in rice germ, cotton seed, commerical oils such as olive oil, and vegetable oils such as crude maize oil. Small quantities are found in wholemeal flour, nuts, legumes and meat, green vegetables; for example cabbage, lettuce, spinach, watercress and alfalfa; and traces in animal products such as butter, milk, eggs, placenta, muscle and sub-cutaneous fats. The quantities found in the viscera, brain, kidneys and testes of animals are almost negligible and Cod-liver-oil and Fish-liver-oils are also almost devoid of it. (Todd 1939.)

Wheat germ oil is a pale yellow viscous oil with an agreeable aromatic odour extracted from the wheat germ, and the unsaponifiable fraction of this oil was used as the raw material for the first chemical investigations on the vitamin. (Evans & Burr 1927). Little advance was made for some years because of the difficulties of fractionating the complex mixture of substances present in the wheat germ oil, and the troublesome biological tests involved in determining the different chemical substances found in it. It was found that on saponification of the oil the fraction left was mainly Sitosterol and that when this had been removed the residue contained a high percentage of Vitamin E.

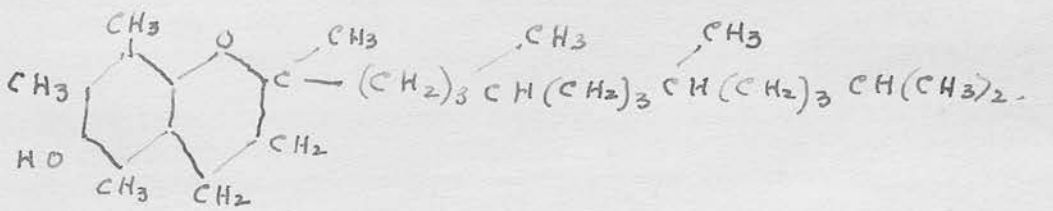
The first real advance was made by Evans, Emerson and Emerson who isolated as crystalline allophanates, two alcohols, α and β tocopherol (Evans, Emerson, Emerson 1936.) tocopherol was found to have the formula C29H5002 and β tocopherol that of $C_{28}H_{48}O_2$. This analysis was done by the x ray crystallographic method (John 1937) α tocopherol was discovered to be active in rats in doses of 2-3 mgms and β tocopherol in doses of 5 mg, allowing reproduction to proceed normally. The relative activity of these two tocopherols was later confirmed by Bacharach who found the mean fertility dose of α tocopherol to be 1.2 mg and of β tocopherol 1.9 mg. The mean fertility dose is that amount which allows 50% of pregnancies to proceed normally (Bacharach 1938c.) . A further alcohol isometric with β tocopherol was isolated from cotton seed oil and termed γ tocopherol (Emerson, Emerson, Evans 1936) and two other tocopherols were

also isolated from wheat germ oil and called Neotocopherol and Cumotocopherol. (Karrar, Salomon, Fritzsche 1937.) (John 1937)

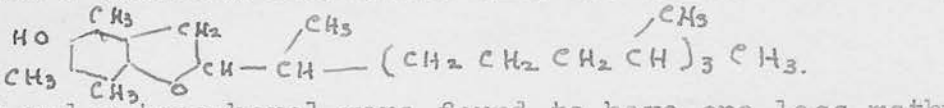
The methods of isolation vary but the one most commonly used is saponification of the wheat germ oil prior to partition between solvents and removal of the sterols with digitonin. After this the oily residue is fractionated by distillation under reduced pressure and, by repeated chromographic analysis, the tocopherols are isolated as crystalline allophonates. (Todd 1939) Some Authors maintain that better results are obtained from direct chromatographic analysis without saponification and whether this be so or not the method is of interest in showing that the tocopherol in wheat germ oil exists in a free state rather than as esters. (Moss. Drummond 1938)

The next advance in the chemical field was the production of duroquinol on pyrolysis of tocopherol at 350°C. This change was also produced by heating the α tocopherol with Selenium. In a similar way ψ Cumoquinol was derived from β tocopherol. (Fernholtz 1937) The production of these quinols and the similarity in their properties and biological effects suggested that α and β tocopherol were simply homologues, differing only in the number of methyl groups on their aromatic nuclei. They were first thought to be mono-ethers of duroquinol and ψ cumoquinol but these substances were found not to have the same properties or spectra as α and β tocopherol and they were also more stable to oxidation. So it was then suggested that they were cyclic ethers and coumoran and chroman derivatives, and these did have similar spectra, reducing properties and behaviour on pyrolysis. (Todd 1939) The derivatives fixed upon were 5 hydroxycoumoran or 6 hydroxychroman with long aliphotic side chains on a heterocyclic nucleus. (Bergel, Todd, Work, 1938). (Danielli 1938).

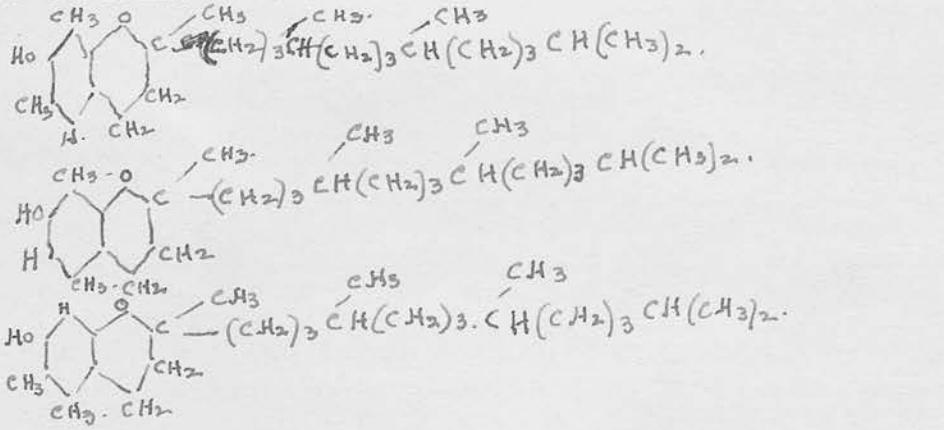
Having reached this stage, the next advance was obviously an attempt to synthesise the Vitamin. This was done by condensing ψ cumoquinol with phytyl bromide in light petroleum in the presence of anhydrous zinc chloride. This produced an optically active substance with similar properties to α tocopherol (Karrar, Fritzche, Ringer, Salomon 1938) (Bergel, Copping, Jacob, Todd, Work.1938). The natural α tocopherol is dextro - rotatory, but the synthetic product is racemic and the relative activities of the natural and laevo-rotatory forms are not known. (Bacharach 1939{c}). By now the evidence was in favour of a chroman rather than a coumaron derivative but it was still ambiguous. Further investigations have all been in favour of α tocopherol being a chroman derivative with the formula -



(Fernholz 1938) Although theoretically it might have been a coumaran derivative with the formula -



β and γ tocopherol were found to have one less methyl group in the aromatic nucleus and each to have the formula of one of three isomers but which are which is not certain. The formulae are -



(Emerson 1938).(Karrer, Fritzche 1938). During this synthesis it was found that the number of methyl groups on the aromatic ring affected the potency, and that for maximum biological activity the structure of the long aliphotic side chain is essential. The most favourable results were obtained with three methyl groups on the aromatic nucleus. (Karrer,Bergel 1939) More recently exceptions have been found to this rule, as although Duro-quinone with no side chain has considerable activity, some substances with side chains approximately similar to that of tocopherol have none. (Evans 1940) It has also been suggested that one part of the molecule of α tocopherol might be responsible for the anti-sterility and another part for the anti-neuritic properties of Vitamin E. In animal experiments Ferric Chloride has been found to destroy the first property but not the second when the Vitamin in the diet has been treated with it and, as it is known that Ferric Chloride oxidises the Vitamin, the above theory may explain this action. Although it may be that a smaller quantity of the Vitamin is needed for the second action as it is only partially destroyed by this treatment.

α tocopherol is stable to heat, there being no loss of biological activity on heating wheat germ oil to 170°c for three hours and it is also stable to dessication, acids, aeration for four hours at 97°c, hydrogenation, and distillation of the most concentrated preparations at 220° - 250° c in vacuo. On the other hand it can be readily oxidised in the presence of alkalis or impurities, being particularly easily decomposed in the presence of rancid fat or lard. It is also unstable towards short wave light, treatment with acetic anhydride, hot saponification and bromination in glacial acetic acid. The esters of tocopherol however were found to be more stable

and for this reason dl α tocopherol or, as it is now known synthetic racemic α tocopherol acetate, has been used extensively in morbid conditions possibly due to Vitamin E deficiency. (Isler 1938).

Some authors found that large doses of a wide range of compounds, such as hydroquinone derivatives bearing no specific relationship to Vitamin E, produced the same effects (John 1939) A possible explanation of this is that they displace tocopherol in the body or that the latter is synthesised from them (Bacharah 1939(a).) More recent investigations have shown that relatively few compounds even as closely related to tocopherol as its oxidative products really possess its properties. An oxidative product α tocoquinone and Naphthotocopherol show slight activity, the latter in doses of 25 mg.. Naphthotocopherol also possesses the properties of Vitamin K and this chemical similarity between Vitamin K and α tocopherol suggests they may have a similar origin in nature and in fact it is found that they do occur together in plants. Tocopherylquinone has no Vitamin E activity although α tocopherol easily undergoes irreversable oxidation to this substance. If this should happen in the normal metabolism of the body it might explain the relatively large curative doses of Vitamin E needed in comparison with other vitamins.

Trimethylphytylbenzoquinone is also inactive and apparently cannot be converted to tocopherol in the animal's body either by reduction or cyclization, which also applies to 2,3,5 - trimethyl - 6 (β γ dihydrophytyl) - 1,4- benzoquinone. Both these last two products are also oxidative derivatives of toxopherol (Tickler, Evans 1941)

Finally quinone and benzoquinone show no Vitamin E activity in doses of 25 mg. and this fact is against the theory that, during metabolism of tocopherol, reductive cleavage might occur to yield biologically active hydroquinones which might function as part of an oxidative-reduction system. (Emerson, Emerson, Evans 1939)

Chemical Tests.

Now that all three tocopherols have been synthesised, chemical methods for estimation of the vitamin have been made possible.

There are four of these methods in use. The best of them was devised by Emmerie and Engel. Ferric chloride is first reduced by the tocopherols and the ferrous salts determined colourimetrically with α:α' dipyridyl. The reduction is carried out in alcoholic solution and the colour of the ferrous-dipyridyl complex measured with a Zeiss-Pulfrich photometer. Comparison is then made with a standard curve previously calculated with dl α tocopherol. This test is best carried out on a saponified preparation of wheat germ oil

and not on the crude oil, as this process removes other non-specific reducing agents which would otherwise affect the result. Saponification is carried out with alkaline potash. (Emmerie, Engel, 1938, 1939.{a}.1939.{b}). Wheat germ concentrates still contain caratenoids although no vitamin A and these should also be removed before this test is applied if an accurate result is desired. It has been found that Floridin X S Earth is the best material for removing Vitamin A and Caratenoids. (Emmerie, Engel 1939.{b}.)

Another test was devised by Furtner and Meyer and depends on oxidation of the tocopherol with nitric acid with the production of a red colour. (Furtner, Meyer 1937) However unlike the above test this one does not differentiate tocopherol from other oxidation products derived from it, although substances differing more widely from it give a yellow instead of a red colour.

Then there is Karrer and Keller's test in which Gold Chloride is reduced at 50°C and followed by electrometric titration. The disadvantages of this test are, that there is no sharp break in the titration, and the complexity of the apparatus needed. (Karrer, Keller 1938).

Finally tocopherol may be estimated by the intensity of the Spectroscopic band in the ultra violet spectrum near 294 mμ but, although sometimes used, this test has not been considered specific enough. (Drummond, Singer, MacWalter 1935). A modification of this test to increase its accuracy can be carried out by noting any selective absorption at 265 mμ and then oxidising the tocopherols with ferric chloride with a result that there is an increase in intensity at 265 mμ, which is five times greater than the intensity produced by the unoxidised tocopherols at 294 mμ. It is therefore easier to assess; this being done after any selective absorption noted at 265 mμ prior to oxidation of the tocopherols has been substracted. (John 1939).

For the same reasons as noted above in Emmerie and Engel's test, these last three tests are best carried out on saponified preparations. No great loss of tocopherol seems to be incurred during saponification (Lester Smith, Bailey 1939), although as much as a 20% loss has been noted by some authors.

Of the chemical tests that of Emmerie and Engel appears to be the best, as it is relatively simple and estimates tocopherol and the reducing substances allied to it which have the same activity. The spectroscopic test is the least accurate as it does not account for these latter substances which do not show a selective absorption in the ultra violet spectrum near

294 mμ as tocopherol doses. (Drummond 1937). None of these tests differentiate between α and β tocopherol.

Emmerie and Engel's test can be used for estimating the tocopherol content of blood serum. 10 ml of serum are treated with dilute alkali in the presence of formaldehyde and Ethyl alcohol. Ether is used to extract the tocopherol and the extracts are washed with alkali and acid. Finally before the estimation of the tocopherol the solution is treated with Floridin Earth to remove the Vitamin A and Caratenoids.

Biological Assay.

Apart from the chemical tests Vitamin E may be estimated by Biological Assay. This was first introduced by Evans and Burr in 1927 who laid down certain uniform conditions to be used, among which was a single oral dose. But it was not until some years later that it began to be used at all extensively. The criteria of response to the assay dose and the relationship between the time of dosage and the response elicited as laid down by Evans and Burr did not give accurate results and made the test one of excessive length. The cause of the latter difficulty was that on experimental production of Vitamin E. Deficient Rats, Investigations found that the animals were able to produce at least one litter before becoming sterile and that it took from fifty to a hundred and fifty days for testis injury to appear. This was apparently due to inadequate removal of Vitamin E. from the diet and to initial storage of the Vitamin during lactation. This was overcome by removing all supplementary sources of Vitamin E. from the mother and suckling's diet during the latter half of pregnancy (Bacharach 1938. ta), and by the use of a breeding diet with very low but adequate Vitamin E. content. (Ringsted 1935). The usual procedure being to use a stock diet containing three times the minimal quantity of the vitamin necessary for the rat and substituting a Vitamin E. deficient diet during the last part of lactation so that the suckling young only get a supply of Vitamin E through the placenta and mammary gland and do not get it through eating their mother's diet. (Mason, Bryan 1938) By this means three-months-old virgin female rats are produced for biological assay which from past experience can be used without preliminary demonstration of a proven resorption, as this supply is not adequate to prevent resorption during the first gestation in offsprings otherwise deprived of Vitamin E. After this treatment testis injury is also found in male rats after only thirtyfive to forty days. This has reduced the time and cost of the test considerably.

First of all Experimenters considered that 100% gestation -resorption being characteristic of Vitamin E. Deficiency, the response to Biological Assay must be an all-or-none one. So

the rats were mated in sufficient numbers and the percentage of live litters following the administration of the Vitamin E was regarded as a function of the dose. The relationship between the percentage response and the dose could be plotted on a curve of the sigmoid type which could be converted to a straight line relationship. (Bacharach 1939.(c).).

More recently it has been suggested that the length of the test could be shortened and its accuracy increased by performing an autopsy before and after removal of the living and dead foetuses and the resorption sites. Then numerical values can be calculated for an uterine index from the following formula - The weight of the uterine contents in grams, added to the number of viable foetuses when two or more are present, and divided by five. Values less than unity indicate a negative response, those below 0.35 being completely negative and those between 0.35 and unity being subminimal. Positive responses give value from one to four and in general are proportional to size of dose. Comparison of the results given at the sixteenth day and at term show a very satisfactory agreement. (Mason 1939.(a).).

No uniformity seems to exist in the time of administration of the assay doses. If given during pregnancy it is best given on the fourth day. The size of the dose of Vitamin E concentrate required on the eighth and fourth day of pregnancy and on the actual day on which evidence of positive mating is obtained is in the ratio of three to four to five. When given before mating it is best given on the eighth day before this and when the dose is given on the fifth, fourteenth and twentysecond day prior to mating it is needed in the ratio of eight to twelve to twenty-four. As there may be incomplete absorption or utilisation from a single dose of the Vitamin especially in those giving responses somewhat above minimal level, the giving of several doses may produce more accurate results, and this has been found to be the case when five doses of molecular distillate of wheat germ oil are given on the fourth to the eighth day of pregnancy after it has been proved that conception has occurred by examination of a vaginal smear. (Mason 1939.(a).).

Animals differ considerably in their response to the vitamin as does the purity and content of the latter in various samples which may have to be tested. So, as the Biological activity of these means the content of tocopherol and not the effect on certain animals, it was necessary to have a standard preparation set up. This removes all but a minimal error and Mason's method as noted above can still be used for comparing sources of Vitamin E with the standard preparation.(Bacharach 1939.(a).). Synthetic racemic α tocopherol acetate was recommended as the substance to be used as this standard. The

investigation on its chemical, physiological and biological properties, the suitability for its adoption for an International Standard, and the manner of its application in biological assay was carried out by the Vitamin E Sub-committee of the Lister Institute and Medical Research Council.

In the calculation of the dose of this International Standard, four solutions of graded strength were used with the object of obtaining a relationship between the dose and the response. This response was defined as the fertility rate which is the percentage of positively mated females which produce a litter. It is thus an all or none response. The dosage-response relationship was plotted as a sigmoid curve and converted into a straight line by plotting the normal equivalent deviation of the percentage response against the logarithm of the dose. Most workers found that the median fertility dose, which enabled 50% of the rate used to produce litters, varied from .56 to 1.71 mgms with an average of 1 mg..

So the International Standard was defined as a unit possessing the Vitamin E activity of 1 mg. of α tocopherol acetate ($C_{31}H_{52}O_3$), which represents the average amount which prevents resorption-gestation in rats deprived of the vitamin when the substance is administered orally. If it is administered parentarily its action is greater. The standard preparation must comply with certain criteria as laid down by the League of Nations such as, in its appearance, specific gravity, refractive index and spectrum. It is issued in the form of a solution in olive oil in 10 gram bottles, one unit being contained in 0.1 grams, and is distributed by the Department of Biological Standards, the National Institute for Medical Research, Hampstead, London, N.W.3. It should be kept in cold storage at a temperature of $0^{\circ}C$ or lower. (Hume 1940).

So at the present time probably the most accurate method of Biological Assay is by using Mason's method in a parallel series of animals, and so finding out the amount of the preparation under test which is equivalent in activity to a known quantity of the Standard preparation. Then the Vitamin E content of the former can be expressed as the number of International Units present in a given weight. Both should be given orally and to find the median effective dose it is recommended that two doses of each should be given not bearing the ratio to one another greater than two to one, and that ten rats should be used for each of these four doses with ten as negative controls.

According to some authors there is satisfactory agreement between the results of the chemical tests and Biological Assay in the evaluation of Vitamin E and the following figures were given in support of this:

Material Used.	Biological Determination of Tocopherol Content per cent.	Chemical Determination of Tocopherol Content per cent.
Wheat Germ Oil.	0.35	0.24
Wheat Germ Oil Concentrate.	29	25
Cotton Seed Oil.	0.21	0.17
Cotton Seed Oil Concentrate.	15	19

(Emmerie, Engel, 1939.(a).).

Although others state that the chemical tests over-estimate the biological activities of concentrates and underestimate those of oils, and do not agree with Biological Assays because the proportions of α and β tocopherol are not constant and the experimental error is not the same in each case. (Lester Smith, Bailey.1939). The experimental error in Biological Assay may be as high as thirty five to fifty per cent. One of the reasons for this is that the minimal dose of Vitamin E for the production of biological activity in animals has not been determined with sufficient accuracy. As has been pointed out the use of an International Standard has rendered this error as small as possible, and if it is ~~possible to~~ proven conclusively that equal amounts of tocopherol and natural Vitamin E, as found by Chemical tests and Biological Assay, have exactly similar effects on experimental Vitamin E deficient rats it may be possible to dispose of the latter.

It has been suggested that rabbits might be used for the biological estimation of Vitamin E as there is a more rapid onset of muscular dystrophy in them than of sterility in rats. (Mackenzie, McCallum.1940).

No evidence of importance on the action of Vitamin E in the body has been derived from its chemistry except that it is unlikely to be a reversible oxidation-reduction process.

THE PHYSIOLOGY OF VITAMIN E.

Possible theories as to the mode of action of Vitamin E in cell metabolism and in the function of the reproductive and nervous systems will be considered in subsequent sections so that only problems of a more general nature, such as absorption, storage and dosage will be considered here.

Absorption.

One of the most popular explanations of the reason why some people should suffer from a vitamin deficiency while others do not, although both taking the same kind of diet, is that its absorption varies in different individuals. If Human Beings really do suffer from a deficiency of Vitamin E this theory must be especially applicable for their case, as few people's diet can be deficient in it owing to its widespread occurrence. Vitamin E must be absorbed along with other fats in the diet so that such factors as lack of bile or fat-splitting enzymes in the intestine will undoubtedly decrease the quantity reaching the tissues. In fact it has been found that feeding desoxycholic acid along with Vitamin E to rats with biliary fistulae increase the absorption of the latter, as, being deprived of the vitamin at a later stage, degeneration of the testes was delayed for a longer time than was usual in these cases. (Graves, Schmidt, 1937). Also a nutritional Muscular Dystrophy and a degeneration of the testes, both of which were similar to that produced in several mammals by eliminating Vitamin E from the diet, were found in dogs with chronic biliary fistulae although maintained on an adequate diet. It is probable that these conditions were due to a deficiency of the vitamin resulting from faulty absorption in the absence of bile in the intestine, and the same factors might account for the extreme muscular weakness found in cases of sprue in Human Beings. (Brinkhouse, Warner-1941).

The co-existence of gastro-intestinal disturbances and syndromes stated to be due to Vitamin E Deficiency have been noted in some cases but it is not a general rule.

In experimental animals fed on large supplies of Vitamin E it is found that a high percentage is passed in the faeces or destroyed in the gut and that little is actually absorbed. It cannot be proved if the same applies to man but it does seem possible that some people are unable to absorb the necessary amount from a normal diet (Currie 1937). Many have suggested that Sterility in mankind might be due to general disturbance of nutrition (Kennedy 1926). (Macomber 1929), but up to date the relationship of so called Vitamin E-Deficiency Diseases in man to defective absorption has yet to be

proved. However it can be said that an individual difference in the ability of various animals to absorb the vitamin would help to explain the divergence in symptoms found.

Diet.

As has been stated above the diet of man can rarely show a deficiency of Vitamin E, and the same can be said of animals except in artificial conditions.

First a few examples of diets used to produce deficiency in experimental animals will be cited. Nelson used the following diet to produce hypophyseal changes in rats.

Casein.	18 parts.
Cornstarch.	54 parts.
Lard.	22 parts.
Cod Liver Oil.	2 parts.
Salts.	4 parts.

0.4 - 0.6 gm of yeast daily. (Nelson.1933).

A somewhat similar diet was used by Barrie in her experiments on rats to show prolongation of gestation and other phenomena produced by Vitamin E Deficiency.

Fat-free Casein.	2000 gms.
Rich starch.	6000 gms.
Lard.	800 gms.
Cod Liver Oil.	200 gms.
Salt Mixture	500 gms.

Yeast extract. (180 I.V. of vitamin B per 100 gms of diet). This diet produced a complete sterility but, if light white casein was used instead of fat-free casein, enough vitamin E was present to allow the rat to produce one or two live litters although the sucklings normally died. (Barrie.1938.(b)).

To estimate the difference in weight produced in the organs of rats by a lack of the vitamin a diet of:

Extracted Casein	25 parts.
Cane sugar	50 parts.
Lard.	10 parts.
Dried Brewer's Yeast.	10 parts.

Steenbock's normal salt mixture 5 parts.
0.2 gms Cod-liver-Oil daily for Vitamins A and D; was used. (Copping, Korenchevsky).

Finally Einarson and Ringsted in their famous experiments on the neuropathic disturbances in adult rats on Vitamin E free diet used the following breeding diet containing sufficient of the vitamin for the mothers to produce normal sucklings, but ~~insufficient for those~~ ones with depleted natural stores of the vitamin.

Skimmed milk powder (Proteins 38% Mineral Salts 9.0% Lactose 48% Butter fat 1.0% Water 4.0%)	30%
Rice flour	40%
Yeast	15%
Non-hardened Cocoanut oil with Cod liver oil	15%

This diet is known as Gudjonsson's diet, Number 4. They also used two diets completely deficient in Vitamin E, any small amount which might be present in other fractions being destroyed by the mixture and storage with the oxidised lard.

Vitamin E. Free Diet 11.

Caseinogen	20%
Rice Starch	53%
Oxidised hardened lard	15%
Dried Yeast	7%
Salt Mixture	5%
Vitamin A.	40 I.U. Daily.
Vitamin D.	2 I.U. Daily.

Vitamin E. Free Diet v.

Caseinogen	20%
Rice Starch	68%
Dried Yeast	7%
Salt Mixture	5%
Vitamin A.	40 I.U. Daily
Vitamin D.	2 I.U. Daily

(Emarson, Ringsted. 1938.).

It has been suggested that rancid lard produces a sterility in rats unrelated to Vitamin E Deficiency and for this reason lard is best excluded from the diets used in experiments on these animals. (Kudyachov. 1933.).

A deficiency of Vitamin E in the natural diet of animals has never been proved but seems unlikely except under artificial conditions owing to its widespread distribution. It has been found that cattle, fed on Millar's Offal or wheat-feed both sources of the vitamin, undergo abortion which is amenable to treatment with it. For this reason it was suggested that the general management, intestinal products or other factors, were more likely to be the operative ones, although unless the food was quite fresh it might have lost its Vitamin E activity before the cattle consumed it. Adamstone has brought forward evidence of a natural occurence of Vitamin E Deficiency in chicks. He found that supposedly normal embryos showed spontaneous haemorrhages and a histological picture apparently identical with that found in experimental Vitamin E Deficient chick embryos. The development of the embryo is dependant on the stores of Vitamin E in the egg and this experiment seems to prove that possibly at a time when high egg production coincides with a lack of green food in the diet, Vitamin E deficiency may occur under apparently normal feeding conditions.

In the case of Human Beings the inference must be the same and in relatively few cases is it found that syndromes stated to be due to a Vitamin E deficiency occur in people who are taking an abnormally poor diet. This is the case in diseases of the Nervous as well as in those of the Reproductive System. (Denker, Scheinsman. 1941.). (Currie. 1937.). (Taylor. 1940.).

On the other hand many Authors state that a deficiency can very easily occur. It was found that diseases which were apparently cured by pure Vitamin E were unaffected by lettuce, spinach, watercress and other foods containing the vitamin, which seems to be in favour of there being little of it in excess of minimal requirements in the average diet. (Shute. 1938.(b).). Bicknall states that the vitamin is only present in milk

from pasture fed cows, that egg yolk may be deficient in it owing to the artificial rearing of the hens, (Barnum.1935.), and that owing to its instability in the presence of certain salts and fats it is probably lacking in imported preserved meat. Also rancidity of butter and margarine may destroy it, as may the cooking of bread. So, as few people eat green leaves and nobody wheat germ oil, he maintains that our diet may be on the edge of a vitamin E deficiency which becomes serious if there is an increased demand or decreased absorption of the vitamin. (Bicknall.1940.). In experiments carried out by Shute it was found that lack of Vitamin E was accompanied by the presence of an antiproteolytic factor in the blood serum, and he states that during the summer, while on a good diet, women in general do not display it and those that do, probably suffer from a specific inability to assimilate the vitamin. Further he found that the greatest number of abortions in hospital practice occured in April and May after the restricted diets of winter and also that rats started on a Vitamin E Deficient diet in late Autumn tended to absorb their foetuses during the same period. (Shute.1936.). As well as in cases of abortion, he found this same seasonal rhythm in the incidence of cases of abruptio placentae and in the conception of deformed foetuses, both of which conditions may be due to lack of the vitamin. The fact that they were rarer during the months from July to September, when the average diet contains abundance of fresh greens, than from January to June, does seem to indicate a natural occurence of Vitamin E deficiency especially in the winter. He also found that it was necessary to treble the average dose of wheat germ oil to prevent abortion in women who started their pregnanceis from January to June. Finally in males he demonstrated an excess of his anti-proteolytic factor in 70% of cases during the months of April to May, but only in 50% during October and November. (Shute.1938.(b).).

Young suggests that the declining birth rate may ~~have~~ be due to changes in the diet with resulting lack of Vitamin E which would thus be of national importance. (Young.1937.). and Rosenberger also thinks that the American diet may be deficient in the vitamin, owing to the modern methods of milling grain and the custom of eating little in the way of green foods, as well as by its destruction during the pasteurisation of milk and in the preservation of meat.(Rosenberger. 1941.). Other authors confirm this natural lack of the vitamin (Drummond.1939.(c).).(Wechsler.1940.(a).).(Barrie.1939.(c).); although some postulate that persons will only suffer from diseases due to its deficiency when there is a constitutional defect of the system concerned so that it requires a greater supply than is usually present in the normal diet. (Einarson, Ringsted.1938.).

Finally on the practical side it has been noticed that in Southern India where the staple diet is milled rice, which is deficient in vitamin E, the incidence

of premature births is three times greater than in Northern India where whole wheat is widely used. (Balfour, Talpade.1932). In contradiction to this it has been found that in this latter district, especially around Lahore, examples of muscular dystrophies are common although this is also supposed to be due to a lack of the same vitamin.(Taylor.1940.) . The difficulty in settling this problem seems to be in the lack of a satisfactory standard of what constitutes normal and subnormal nutrition as far as this vitamin is concerned, and until this is determined it is impossible to assess the nutritional state of various individuals.

Dosage.

There are no definite standards for the daily requirements of vitamin E in experimental animals or Human Beings so that they can only be assessed by the success or otherwise of the dosage given in a particular case.

In cases of Habitual Abortion in rats the minimum effective dose has been found to be 15-30 mg. daily or 300 - 600 mg. of Wheat Germ Oil as a single dose, as this results in the completion of any particular gestation. (Evans, Burr.1927.) . After repeated abortions or absorptions it has been found that the requirement of experimental animals for the vitamin increases. It has been suggested that this might be due to the degenerative pigmentation of the uterine muscle. (Barrie.1939.(a)). Apart from this there might also be an endocrine disturbance resulting in an increased obstacle to implantation. Working on this theory Bacharach found that the dose of Vitamin E necessary to produce 50% fertility in animals with one previous abortion was four to ten times greater than in virgins. (Bacharach. 1939. c.). Barrie also found that although .35 ml. of a vitamin E preparation were effective in virgins, .75 ml. had no effect in rats with previous resorptions. Finally it has been found that the amount of the vitamin necessary for the delivery of litters of normal size is four times that required for the delivery of a single foetus, and that the amount necessary for normal lactation is at least three times the latter.

When treating similar conditions in human beings one can only give an equivalent dose to that found effective in animals. Currie found that 5 mg. of tocopherol given to a 200 gm. rat invariably resulted in fertility, so in proportion a 50 Kg. woman would need 1⅔ gms. or 10 mg. daily for 150 days. As some would be derived from the body stores, a smaller dose than this would probably be sufficient although factors such as variations in absorption and utilisation would have to be considered. (Currie, 1939.). Some authors have used more than this, and some less. Vogt Muller, who was the first to give the vitamin in this field, administered 2-3 gms. of wheat germ daily (Vogt Muller.1933.(b).

Collins states that the daily requirements of α tocopherol are probably 2 mg. daily except in later pregnancy when they would be increased to 3 mg. (Collins, Weed, Collins. 1940). Watson gave one dram of wheat germ oil daily to his cases of Habitual Abortion with apparent success. (Watson. 1936). Clark recommends 3 mg. of α tocopherol daily, (Clark. 1940); Dunlop one capsule of Fertilol or Vitealin or one tablet of Ephynal three times a day throughout pregnancy, (Dunlop, Davidson. 1940); and Eddy 3 to 6 c.c. of Wheat Germ Oil in all cases of habitual abortion as soon as pregnancy is recognised and continued throughout it, being increased up to 20 c.c. if threatened abortion supervenes. (Eddy, Daldorf. 1941). Shute found that a single massive dose of 6 drams of Wheat Germ Oil followed by 4 drams three times a day on the first day and 1 drm three times a day on subsequent days, cured cases of threatened abortion and caused the disappearance for as long as treatment is continued, of his anti-proteolytic factor from the serum. (Shute. 1938b).

Patients may require more of the vitamin at one time than another and this must be judged by the physician. Shute states that the dose required increases during pregnancy as the placenta grows, and that it varies with the season and the cyclic phenomena of women. He found that the daily doses required to prevent the appearance of this factor in the serum were one, two and three drams respectively in the three trimesters of pregnancy, and in non-pregnant women none was required from July to September, one dram from October to June and two to three drams from February to June. He also noted that some women tend to abort or miscarry at times when ovulation or the menses would have occurred and recommended that the dose be increased at these times. He reported that three ounces of Wheat Germ Oil daily could be given without harmful effects. Hypothyroids seemed to require more of the vitamin than normal, possibly due to the defective excretion of oestrogen but the theory of this will be discussed later. (Shute, 1939).

Finally it has been found that some cases of the disturbance of the reproductive mechanism failed to respond till the dose administered is raised past a certain level, which led to the theory that in these cases the vitamin acted on an all-or-none principle and this might account for the apparent failure of some author's results.

The same factors apply to the treatment of pathological conditions of the nervous system stated to be due to vitamin E deficiency. Apparently to prevent muscular dystrophy in rabbits the dose of α tocopherol does not need to exceed 1 mg. per kilo body weight per day, (Mackenzie, McCallum. 1940), and in suckling rats it is prevented by 10 mg. of α tocopherol to the mother on the day of littering or 1 mg. daily to the young from the 10th day or 3 mgs. daily from the 15th day. A similar dose from the 16th day is ineffective. (Evans, Emerson. 1940). .0075 mg. of dl-α-tocopherol per gram body weight per day prevented the symptoms of encephalomalacia in chicks. (Dam, Glavind, Bernth, Hagins. 1938).

Wechsler found that 7 to 1 milligrams of tocopherol per kilo body weight were necessary to prevent nervous symptoms in experimental animals, so in proportion he treated his human cases with 50 mg. daily as an average. He also noted that unless the cause of the failure of the adequate supply of the vitamin is found the patients may have to be treated permanently. (Wechsler. 1940. b.). Later he states that the usual dose given in these conditions is too low, and that he has given as much as 1000 mg. of tocopherol daily, partly orally and partly intramuscularily. He found that cases of Amyotrophic Lateral Sclerosis which did not improve on 100 mg. of tocopherol daily, began to do so on 150 - 300 mg. intramuscularily and 200 mg. or more by mouth. (Wechsler. 1941.). Fitzgerald gave to cases of motor neurone disease and muscular dystrophy a daily average of 18-36 mg. of α tocopherol, plus 300 mg. of dl-α-tocopherol acetate in some cases but without success. (Fitzgerald, McArdle. 1941.). Beaumont and Dodds recommend $\frac{1}{2}$ oz. of fresh dried whole wheat germ or 6 mg. capsules of tocopherol or 3 mg. of dl-α-tocopherol acetate by mouth, or 1 mg. of the latter intravenously twice a day. (Beaumont, Dodds. 1941.). Possibly the vitamin should always be given parentally as well as orally in case its defective absorption from the gut has been the primary cause of the condition under treatment.

Preparations of the Vitamin.

Vitamin E can be given in various forms to Human Beings: First as dried fresh wheat germ or as wheat germ oil, which has a low and variable content of the vitamin, but may contain water soluble factors necessary for the prevention of pathological lesions of the nervous system. To preserve the activity of the oil it must be kept in the cold as, if it is not, the activity is destroyed and the symptoms return, and at no time does it retain a reliable potency for more than eight weeks and possibly only for a few days at room temperature. No efforts have been made to find a preservative for the oil. (Shute. 1938. b.). Other authors have found that if it is kept in a vacuum as well as at a low temperature, it may remain active for as long as eight months. (Palmer. 1937.). It takes 21 days to standardise wheat germ oil on rats, so this method is not used as only five weeks of potency would be left. (Shute. 1938b.).

Secondly the vitamin may be given as a concentrated preparation of α tocopherol or of dl-α-tocopherol acetate made up either in capsules or tablets and taken orally, or as a sterile solution given intramuscularily or intravenously. Many of these are on the market and a list of the commoner ones will be given:-

Davitamin E. (Organon Laboratories. London.).
5 millilitres wheat germ oil.

Ephynal. (Roche Products.).
3 milligrams synthetic dl-α-tocopherol acetate per tablet.

Fertilol. (Vitamin Ltd. London.).
3-5 minims Wheat Germ Oil per capsule daily for at least three months.

Germinal.(Paines and Byrne, Ltd.).
Wheat Germ Concentrate. 1 millilitre ampules equivalent to 100 grams of Wheat Germ. Capsules equivalent to 5 grams of Wheat Germ Oil.

Phytopherol . (British Drug Houses. London.).
3 millilitres of oily concentrate, containing 3 milligrams of dl- α-tocopherol acetate.

Profecundin . (Richter. London.).

30-40 drops of 12 per cent solution of Vitamin E or two tablets of 0.01 grams of Vitamin E three times a day.

Trigol. (Abbott Laboratories. London.).
Wheat Germ Oil preparation.

Wheat Germ Oil - Callosal Brand.(British Colloids Ltd. London.).
3 minim capsules.(0.2 grams of Wheat Germ Oil). The Vitamin E potency being 40 units as expressed on the Pacini-Linn scale. i.e. 25 milligrams daily is required to ensure a litter of rats from Vitamin E depleted mother.

Viteolin Capsules.(Glaxo Laboratories. London.).

3 minim capsules - The unsaponifiable matter from 5 grams of Wheat Germ Oil, containing 6 milligrams of tocopherol.
Zygon. (Squibb. New York., Savory and Moore.London.). Wheat Germ Oil or 3 minim capsules. Dose of one teaspoonful or six capsules daily.

Storage.

Authors seem to be agreed that very little Vitamin E is stored in the animal body. This may be due to the ease with which the aliphatic ring is opened in the body, resulting in its destruction.(Drummond.1939.).

What little is stored is soon used up by the normal metabolic processes when the diet is deficient in the vitamin, and no experimental animal retains its fertility after three to four months when completely deprived of it. On the other hand a female rat does possess sufficient stores to complete pregnancy and rear a normal litter as this is accomplished if a normal rat is given an E Deficient Diet from the day of mating onwards. Young rats whether reared by normal or E Deficient mothers, have very small stores of the vitamin which are insufficient to carry it through the suckling period, as, if it is denied them from birth, they develop paralyses about the 18th day which is cured by the vitamin if given soon after the symptoms appear. (Barrie.1938c.).

Vitamin E is found mainly in the muscles and body fat of the animal, but none is found in the unsaponifiable extracts of the heart, blood, kidneys, adrenals, testes,

ovaries, liver and pituitary. (Cuthbertson, Ridgeway, Drummond, 1940.). The intraperitoneal and subcutaneous fat seems to be the richest store but even here there is only an amount about equal to the daily intake. So it is seen that the vitamin is extremely easily destroyed in the body. A rat given a single dose of 60 mg. of dl-α-tocohperol and killed forty eight hours later shows no trace of it in its tissues, and, if given 3 mg. of tocopherol per week for three months, no equivalent amount can be found in the body fats. It may be noted here that the stores of vitamin A in the liver are greater when Vitamin E is present than when it is absent in the diet. (Moore, Martin, Rajagopal. 1939.). No clue as to the precise function of the vitamin in the tissues has resulted from these studies, although it has been noted that it is used up by the metabolic processes at almost the same rate in pregnant as in non-pregnant animals. (Evans, Burr. 1927.). It has also been suggested that there are larger stores in the female than in the male, so that spermatogenisis is quickly affected, whereas the female sex cycle proceeds unaffected up to a certain stage. There may also be an individual variation in the capacity to store it and this would help to explain the divergence in the time of onset of the symptoms seen in E Deficient experimental animals. (Stone, Manchester, 1941.).

Supply of the Vitamin through the Placenta and Mammary Gland.

The small stores of the vitamin present in the suckling animal must either be derived through the placenta or from the mammary gland. This question became of importance in producing animals so deficient in the vitamin that they could at once be used for its Biological Assay. So experiments were carried out to find which of the two routes was of most importance. First the young were allowed access to the maternal diet containing various quantities of the vitamin in it, and then completely deprived of it, and the delay in the onset of sterility was found to be proportional to the vitamin content of ~~the first~~ this diet. They they were denied access to this diet so that they only got the vitamin from the above two sources, and the mothers were given diets containing three, ten and fifteen times the minimal vitamin E requirements. Symptoms of deficiency were only seen in the offsprings of those receiving the most deficient diet. On the other hand if the young of normal rats are suckled by females on E free diets, all without exception showed signs of deficiency. This shows that the mammary gland is almost entirely responsible for the transfer of the vitamin to the young; but not enough can pass through either channel to prevent resorption during the first gestation of female offsprings, otherwise deprived of it; or protect male ones from testicular degeneration for more than seventy to eighty days. In this way by depriving the mother and the young of Vitamin E in the diet rats can be produced which invariably resorb during the first gestation, even after receiving a dose twenty times the minimal requirement for a normal rat. (Mason, Bryan. 1938.). (Mason. 1939b.).

Requirements and Utilisation of the Vitamin.

Little is known as to the requirements of the animal body for Vitamin E but greater quantities seem to be needed during infancy, adolescence and pregnancy. Some Experimentors maintain that the rate of utilisation of Vitamin E is not increased by gestation. (Evans, Burr. 1927). It does not seem to be necessary to the normal metabolism of some animals such as the goat who may be able to synthesise it, and individual variation in requirements among a single species of animal are large. Shute maintains that the deficiency is accompanied by the presence of an antiproteolytic oestrogenic factor in the blood serum and its disappearance will denote that the requirements of the animal have been met. (Shute. 1938b). Animals with hypothyroidism require greater quantities, However no definite facts on the utalisation of the vitamin have been discovered, although the fact that hypoplasia of the Thyroid can be produced in rats by injecting oestrin fits in with Shute's theory mentioned above. (Benazzi. 1933). He also states that in some cases he can free the serum of his oestrogenic factor by thyroid extracts alone, and explains that these animals may be unable to excrete or may manufacture an excess of the former (Shute. 1938b). Finally it has been noted that hyper-thyroidism in rats led to increased gonad-stimulating power of the pituitary, and that more oestrogenic substance than normal was needed to produce oestrus. (Ham. 1933). Signs of hypothyroidism have also been noted in E Deficient rats. (Barrie. 1937b). However the link between Vitamin E, oestrogens and the thyroid is no more than hypothysis and this will be discussed in detail later. Experiments suggest that the requirements are greater in the female than in the male, as signs of Vitamin E Deficiency are less common in the latter; although it has been suggested that there are separate factors for the two sexes. (Martino. 1934). (Follen, Shuster. 1934).

This individual variation in requirements has been brought forward to explain the divergence of results found by different workers. It has been suggested that some demand a greater concentration of the vitamin than others, (Currie. 1937), or that they are unable to utilise, or inactivate the vitamin in some way. (Watson. 1936). Finally Einerson and Ringsted state that certain people are subjects of a constitutional liability of the nervous system ~~the~~ to require a greater supply of the vitamin than normal and that this exerts itself when the body is exposed to noxious agencies such as infection intoxication or trauma. This liability may be familial or individual. (Einarson, Ringsted. 1938). Similar individual variations in the need for other vitamins have been noted in cases of scurvy, (Moncrieff. 1936), and of rickets. (Thatcher. 1938). Further theories on the role of Vitamin E in the function of the reproductive and nervous systems as well as in cell metabolism will be reveiwed in their appropriate sections.

Excess of Vitamin E.

It has been found from experiments that excess of Vitamin E does not increase the fertility of experimental animals above normal, and has no effect on the course of pregnancy. (Shute. 1936). However storage only seems to occur when there is excess of the vitamin in the diet. No toxic symptoms have been noted in rats or human beings on large doses of the vitamin (Watson, Rew. 1935).

An increase in the size of litters of experimental rabbits fed on large doses of the vitamin has been noted. Other authors deny this. (Evans, Burr. 1927).

Hypersensitivity to Vitamin E.

Several cases have been noted which showed signs of hypersensitivity on being treated with Vitamin E preparations, but whether this was due to the vitamin itself or to impurities in the preparation used is uncertain. Shute records examples in five adults and one infant. The first adult had a history of asthma and hay fever and had been taking 4 drams of wheat germ oil for 14 days. She complained of flatulence, nausea and vomiting. The second was a case of vaginitis, who had received a massive dose of wheat germ oil followed by 2 drams daily, and complained of waves of heat and a generalised urticarial skin eruption. The third had no history of allergy but gave a positive skin test to wheat germ oil. Her symptoms were hot flushes and a generalised, raised, blotching and discrete rash which was very itchy. The fourth and fifth cases also showed an itchy urticarial rash. These symptoms disappeared on cessation of the wheat germ oil treatment. In the case of the infant, its mother was receiving wheat germ oil to help lactation, and as long as this was continued the child had soft yellow stools containing minute bubbles. The stools were normal two days after the mother stopped taking the oil. (Shute. 1938b). De Jong also noted erysipeloid and urticarial reactions in some of his cases and mentions one case of pregnancy toxaemia and one of haematuria which he states may have been due to hypersensitivity to the vitamin. In none of these cases was the dose of the preparation a large one so these symptoms cannot have been a true toxic reaction. (De Jong. 1941).

Excretion.

Traces of tocopherol are found in the faeces but it is not known if it is excreted into the gut or if these are merely the result of incomplete absorption. However, faeces of animals on a vitaminE free diet when extracted with ether and light petroleum show no tocopherol in the petroleum soluble factor on spectroscopic examination, which suggests it is not excreted.

Only when very high doses of the vitamin are given is it found in the urine and then only in minute

quantities. (Drummond.1939a.) . If 3.5 milligrams of tocopherol are given to an animal daily for a week no tocopherol is found in the urine, but three to fifteen per cent of the total amount given in the week, is found in the faeces. Greater amounts are found in the faeces of males than females. On the other hand if the animals are fed on diets rich in Vitamin E for a year, twentyfive per cent of the tocopherol ingested is found in the faeces and small traces in the urine as well. (Cuthbertson,Ridgeway, Drummond. 1940.).

THE SYNDROMES PRODUCED BY THE LACK OF VITAMIN E IN THE DIETS OF ANIMALS AND THE RELATIONS WHICH THESE BEAR TO SIMILAR CONDITIONS FOUND IN CLINICAL MEDICINE.

A. The effect of Vitamin E Deficiency on Cell Metabolism.

(a) The Relation of Vitamin E to the cell nucleus.

It has been suggested that Vitamin E is necessary for the normal division of all cells.(Eddy, Daldorf.1941.). However experimental evidence in support of this is not conclusive, but certain cells do seem to require the vitamin for their normal function. When it is absent areas of rapid cell proliferation may show a more profound change than with lack of other vitamins. There is a general retardation of cell division and they suffer irreparable damage. So the vitamin may possibly be concerned with metabolic processes which are maintained at a higher level in rapidly dividing cells, or it may be essential to a specific and vital cellular function related to mitotic division or other nuclear activity.(Mason.1933a.). (Mason.1939b.).(Juhász-Shaffer.1931d.).(Juhász-Shaffer.1933.) . Mason states from his experimental data that it is more related to processes of cell maturation and differentiation than to mitotic activity. Studies on the rate of growth and cytological characteristics of the Walker 256 mammary carcinoma showed that they were unaffected when monthly transplants were carried out in Vitamin E deficient rats for more than two years. (Mason.1939b.).

If Vitamin E is related to nuclear function in all cells the histio-pathological changes, observed in the germinal epithelium of the testis, the developing embryo and the neuro-muscular tissue of suckling rats, must represent an acute manifestation of a deficiency disease in tissues whose vitamin E requirements are very high at the time of involvement. Delayed growth in later life, uterine pigmentation and changes in the cells of the skin, kidney, thyroid and pituitary may also be a direct or indirect result of chronic Vitamin E Deficiency.(Bacharach.1939a.).

Other Authors have laid stress on the relation of the vitamin to the nuclear chromatin.(Drummond.1939a.). From studies of the maldevelopment of the haemopaetic system in the embryo, and of the muscular system in the suckling and adult rat, as well as of degeneration of the testis in Vitamin E deficiency, this seems the most likely explanation of its cytological function. It may either be necessary for the normal physio-chemical structure of the nuclear chromatin or ~~with~~for the metabolic processes involved in the synthesis of the chromatin molecule, and act directly or indirectly as a kind of morphological hormone in the development of these tissues. (Vogt-Müller.1939.).

The injury to the cell is more suggestive of a nuclear rather than a cytoplasmic derangement as the structural alterations in the testes of E Deficient animals anyhow, are preceded by a fundamental physiological disturbance, so that even if the supply of the vitamin is renewed at an earlystage many apparently normal cells continue to degenerate. Also liquifaction of the chromatin of the nucleus usually precedes alterations in the cytoplasm of the cells. That there may also be alterations in the cytoplasm is suggested by the manner in which the degenerate cells fuse together. This alteration in the physiological state of the nucleus and increased affinity of its chromatin material for iron haemotoxylin suggests a change in the nuclei acid component of the chromatin. The chromalysis has not been demonstrated in the cells of the embryo or central nervous system as yet. (Mason.1933a.).

Further details of the histology of the testes, developing embryo and central nervous system in Vitamin E deficient animals will be given in their appropriate sections, but it may be mentioned here for completeness that it is the mesodermal cells of the rat embryo which seem particularly to need the vitamin. The blood islands and small vessels fail to develop in this layer and the yolk sac and allantors are also abnormal which results in starvation and asphyxiation of the embryo. (Mason.1933a.).(Viner.1931), (Evans,& Burr.1927).

In opposition to these theories of retarded development in the cells of Vitamin E deficient animals, Adamstone maintains that it has a controlling influence on cell division and that when it is lacking certain cells show an excessive proliferation. He does not maintain that this is universal among all cells, the converse being true especially of those of the testes.(Adamstone, Card. 1934). In rats the placenta of Vitamin E deficient females are thicker than normal and in pseudo-pregnant female rats spontaneous deciduomata are commoner than normal. (Evans,1928b.). Davison and other Authors were able to change the character of certain cells by depriving them of vitamin E. and cause them to destroy neighbouring ones. (Davison.1937). Finally Adamstone's studies on chicks may be mentioned. He carried out many experiments on the effect of Vitamin E deficiency on the developing chick embryo and the

growing chick
~~adult hen~~ and noted that the tissues showed signs of uncontrolled proliferation. In the latter, lesions were demonstrated in the visceral organs. Histologically they were found to be foci of degeneration and destruction of normal tissues, accompanied by replacement and invasion by new cell growth. This growth appeared to be derived from an undifferentiated type of tissue having the form of a delicate reticular syncytium. The condition seemed to be one of uncontrolled cell division stimulating malignancy. (Adamstone. 1931, ~~adamstone~~ 1934.). In the former he found that the chicks died at an early stage of incubation if no Vitamin E was present. The cause of this was apparently an early retardation of growth and differentiation with an unrestricted division of certain cells. On the fourth day of incubation there was a disintegration of the blood vessels of the blastoderm and haemorrhages into the caelom and exocaelom. A dense ridge-like 'lethal ring' of cells also formed in the blastoderm and surrounded the embryo, preventing ~~in~~ the expansion of the exocaelom and allantois and interrupting the vitelline circulation. No explanation has been offered as to why the Vitamin E deficient rat embryo should show underdevelopment of the mesoderm and a similar chick embryo shows signs of uncontrolled cell proliferation in this region. The treatment of the diet with ferric chloride to destroy the vitamin, which was used in these experiments, may have some other side action which alters the response of the embryo. (Adamstone. 1931.). However the possible relationship of the vitamin to malignancy will be discussed later.

To complete this section it may be mentioned that it has been found in rats that, if the fathers are deprived of Vitamin E, the sex ratio in the offsprings is eighty males to a hundred females. On the other hand if the fathers have an unlimited supply, the ratio is a hundred and tem males to a hundred females, while if the supplies of the father and mother are equal, it is a hundred and thirty males to a hundred females. The suggested explanation is that there is a difference in the viability of the sex-determining chromasomes due to nutritional changes in the Vitamin E supply of the nuclear material surrounding them. (Pacini. 1935.).

(b) <u>The Relation of Vitamin E to the Oxidation-Reduction processes of the Cell.</u>

One of the theories on the mode of action of Vitamin E is that it plays a part in the oxidation-reduction processes of the cell, especially among the fats (Windaus . 1938.). In support of this auto-oxidants or inhibitols are found in natural oils and fats related to tocopherol. (Drummond .1939.). In opposition to the view of a reversible quinol-quinone system originating in the tissues from tocopherol, oxidation of α tocopherol results in quinone which is an inactive substance in regard to the biological

properties of the vitamin.(Todd.1939.). It is not possible to substitute quinone for Vitamin E in the diet,(Karrer, Salomon, Fritsche.1938.), and it is not detected spectroscopically in animal tissues.(Wright, Drummond.1940.). Also there is no evidence that the extent of oxidative processes in the organism, or the general metabolism, is lowered by deprivation of Vitamin E.;(Evans,Emerson, Telford.1938.), or that the oxygen uptake by isolated tissue removed from animals deficient in the vitamin is depressed.(Drummond.1939.). In fact the uptake has been noted to be raised in some cases.(Madsen,1937).

Another possible relation to the physiological processes of the cell is an anti-oxygenic one. It might protect the cell against harmful auto-oxidising fatty acids. (Mattill, Stone, 1923.). Although it is certain that wheat germ oil does contain antioxidants, (Broadway,Mattill.1934.), they may be mostly other substances than tocopherol. In fact some authors say that Vitamin E is itself dependent on these for protection from oxidation and that they are always in close association with it in the unsaponifiable fraction of fats. (Mattill, Crawford.1930.). Wheat germ oil does protect Vitamin E against oxidation in the presence of ferrous sulphate. (McCallum, Simmonds, Beeker.1925.) However a correlation between the susceptability of fats to oxidation and their content of Vitamin E has been noted. (Cummings, Mattill.1931.).

Treatment of the diet with ferric chloride, which some experimentors used to destroy Vitamin E, may not have had a direct action on the vitamin, but destroyed these antioxidants and so indirectly resulted in its destruction.

It is unlikely that this antioxygenic action is the complete explanation of the function of Vitamin E, as γ tocopherol is the most effective antioxident while α tocopherol has a greater power to cure states of Vitamin E deficiency than either β or γ tocopherol, so that there is no direct relationship between the two actions.(Mattill.1938.).(Olcott, Emerson.1937.); Although there is no reason why this protective action, which has been proved to take place in the diet, should not extend to the physiological processes of the organism. Prolonged exposure to autoxidising fatty acids and their products may be as damaging to their chemically sensitive constituents as it is to the autoxidizable constituents of an experimental diet. This is also supported by the fact that Vitamin E deficient rats could not use large stores of Vitamin E for four to six weeks after transfer to a normal diet (Waddell, Steenbock. 1931.). Mattill suggested that the muscular dystrophy which Experimentors have observed to be produced by Cod-liver-oil in herbivora and suggested was due to some unknown toxic factor in the oil, may be due to the fact that the food remains a long time in their large caecum, due to the presence of the oil and that the vitamin E may undergo autoxidation there. (Mattill.1939.).

The one factor which does give some support to these theories is the ease with which tocopherol is

oxidised both in vitro and in vivo, but apart from this there is little to support them.

(c) The Effect of Vitamin E deficiency on Cell Pathology, with special reference to its Relation to Malignancy and the Haemopoetic System.

It has already been suggested that Vitamin E may be of importance in the cellular processes of the body as a whole or just of particular tissues such as the gonads, pituitary or the developing embryo. (Mason, 1935.). (Adamstone, 1934.). (Drummond. 1939a.). In this section attention will be focused on pathological conditions possibly resulting from a deficiency of the vitamin in these processes.

Details of the histological changes occuring in the male and female reproductive systems, the developing embryo and the neuromuscular system of experimental Vitamin E deficient animals will be considered later in their appropriate sections. There is no doubt that these conditions are produced by lack of Vitamin E but there is no conclusive evidence that it is a simple disturbed function of the cells of the systems, which is the primary cause. (Underhill. 1939.). Here only conditions such as malignancy, which are the obvious results of pathological changes in the cells will be discussed.

Adamstone's observations on the developing chick embryo in the absence of Vitamin E seemed to indicate that unrestricted cell division was taking place, so that a possibility of a link between Vitamin E deficiency and simple and malignant tumours was raised. As has been noted before he found that differentiation of the body structures was practically normal but that beginning within the first twenty four hours of incubation and increasing progressively as the period of hatching advanced, the embryo failed to develop. It normally died at the end of the fourth day or shortly afterwards, either because of failure of development or degeneration of the blood vascular system, or because at a slightly later date there appeared a lethal ring of cells in the blastoderm. This ring entirely surrounds the embryo, and shows intensive cell proliferation in the mesoderm of the blastoderm, while the endoderm is disintegrating. It kills the embryo by choking the developing circulatory system by interrupting the capillary anastomoses between the vitelline arteries and veins, and also results in constriction of the exocoelom which prevents the allantois from developing. The actual cells of the ring are modified histiologically from those which normally make up the germ layers of the blastoderm. More rarely death occured from haemorrhage or malformations. The site of the haemorrhages were marked by the presence of histiocytic mesenchyme cells, which may be involved in the production of the haemorrhage by eroding neighbouring tissues and are probably derived from modified mesenchyme cells of the coelomic lining. (Adamstone. 1931.). The cells are grouped around the rupture in rosette like clusters and have densely pyknotic nuclei and clear protoplasm. It must be considered that they may have exactly the opposite function of stopping the haemorrhage but the

much time to collect and they are not found in the blood stream. Also the histiological evidence does seem to indicate that they accumulate in certain parts of the coelom and implant themselves by eroding adjacent tissues causing haemorrhages as a complication. It is not certain if Vitamin E is the sole factor in producing these changes, (Adamstone.1941c), but they do seem to indicate that cellular multiplication is not being controlled as it should be.

Adamstone then carried out further experiments to find if a closer connection with malignancy could be found. As has been mentioned above he described signs of unrestricted cell division in the viscera of Vitamin E deficient chicks, (Adamstone.1934), and further details can now be given of these findings. He fed newly hatched chicks on diets treated with ferric chloride to destroy the vitamin, and from observation of controls and from consideration of factors, such as the effect of ferric chloride, age, susceptibility of individual chicks, use of synthetic rations and Vitamin E storage and reserves, decided that the lack of the vitamin was the cause underlying the development of the conditions to be described. He admits that it is not possible to rule out entirely the concurrent lowering of the vitamin A supply or the existence of some other causative agent effective only in the presence of Vitamin E.

After prolonged feeding the chicks normally showed definite symptoms of illness with rough feathers, drooping wings and paleness of the comb and wattles. The appetite becomes poor and the chick becomes emaciated, with a disinclination to move about due to lack of balance. Just before death there were pronounced tremors and a sudden drop in body temperature. In a few cases there were no symptoms till sudden death of the bird occured.

On Autopsy thirtyone out of thirtyfour chicks showed definite visceral lesions. They took the form of white creamy or greyish spots in the affected organs. The liver is most seriously affected, but lesions are also found in the heart, pancreas, gizzard spleen, lungs and kidneys. Gelatinous tissue masses were also found in a few cases. Histiological examination of these spots revealed that the normal tissue was destroyed and replaced by a characteristic delicate cytoplasmic reticulum interspersed with small round densely staining nuclei. A small amount of fibrous tissue sometimes occured in the lesions. Large accumulations of lymphocytes were often associated with the reticular tissue, being sometimes so numerous as to crowd everything else out. The lesions are not walled off from the normal tissue and advance into it by the capillary sinusoids. In many cases the general infiltration of the liver was suggestive of a leukaemic involvement.

The normal liver cells are destroyed and disintegrate,

the nuclei becoming pyknotic before final destruction; and the reticular tissue and lymphocytes increase by cell proliferation, many mitotic figures being seen in some lesions while in others the nuclei show simple constriction suggestive of amitotic division. The heart was usually enlarged and showed extensive destruction and invasion of the ventricular wall, the muscle being replaced by reticular tissue and lymphocytes.

The gelatinous tissue growths mentioned above represent great hypertrophy of the reticular tissue and lymphocytes, and continuity can be demonstrated between it and the more normal reticular tissue, the latter undergoing myxomatous degeneration when insufficient nourishment is available.

In the liver the reticular tissue is probably derived from the endothelial wall of the blood sinusoids, in the heart and gizzard it may be related to the loose connective tissue between the muscle fibres, and in the spleen it is similar to the framework of that organ. So it is very likely that the reticule-endothelial cells are the real source of this reticular tissue as they are widely distributed in the body particularly in the liver, spleen, and in close association with loose connective tissue. The lymphocytes seem to accumulate by migration into the degenerating areas.

Adamstone states that the spread of this special tissue may be by independent foci, direct invasion or by metastases. He defines the growth as a neoplasm because of the destruction of the normal tissue, the rapid cell increase by mitoses, the aberration of the lesion tissue from the normal, and the similarity of the basic reticular tissue in all lesions. He also suggests that it is malignant because of the destruction of normal tissue, the diversion of food material from the latter and the occurence of haemorrhages by invasion of the blood vessels. It also seems to be of a severe type as death soon occurs and there is a large divergence from the normal cell structures which is said to be a measure of malignancy.

On the basis of its structure of a stroma of reticular tissue with accumulations of lymphocytes, the growth may be classified as a lymphoblastoma or lymphosarcoma although it bears some similarity to leukaemia in fowls. (Adamstone.1936).

Adamstone also noted in these same chicks, that, if they were given Cod-liver-oil or sardine-liver-oil as a source of Vitamins A and D, they developed ulcers in their intestines which were associated with reticular-cell sarcoma. Whether these tumours were due to ingestion of these oils when the diet was deficient in Vitamin E, or whether it was due to lack of an unknown substance not present in these oils, or to a disturbance in the utilisation of Vitamin D under conditions of Vitamin E deficiency is uncertain. (Adamstone.1941f.).

Fibrosis of the uterus going on to typical fibromyomata has been noted in Vitamin E deficient female rats, and it may be pointed out here that this change proves that the reversibility of sterility in these animals is not absolutely true, and so if they are being used for biological assay, they should be used before these changes occur. (Barrie.1938d.). A similar condition arises after injections of oestrogens, (Lacassogne.1935.), which fits in with Shute's theory that Vitamin E. deficiency is associated with a raised blood-oestrogen content.

A direct relationship between Vitamin E deficiency in rats and the occurence of spontaneous deciduomata has been cited but no explanation given for this. (Evans.1928a.).

Bearing these experiments in mind it might be expected that Vitamin E would raise the threshold of resistance to experimental tumours in animals and this has been found sometimes to be the case. When tumours were produced in mice by tar, those on a high vitamin E diet showed retarded growth of the tumour and an increased length of life. The growths were small and warty with little or no thickening of the tissue at the basic attachment, which is usually thick and indurated. Some become quiescent and remain so, while others even disappear altogether. Infection of the growth which is common as a rule was rarely seen in these cases. Finally if mice on a low vitamin diet, and exhibiting tumours, were switched to a diet rich in vitamins, the growths become dark on the surface and the indurated tissue at the base becomes soft and easily detached, leaving a raw surface with apparent attempts at healing at the surrounding edges. Breeding and vitamins A.B. and E, which are associated with reproduction, all seem to help in building up a resistance to the development of these tar carcinoma. (Davidson.1937.). On the other hand the addition of wheat germ to the diet of mice had no effect on the growth of benzpyrene tumours, (Haddow, Russell.1937.), and Vitamin E did not reduce the incidence of cancer in mice treated with benzanthracene, (Camerson, Meltzer.1937.), nor did its deficiency or excess alter the development of sarcoma in rats. (Marchesi.1933.). Shute treated four cases of Gastric Carcinoma in Humans with wheat germ oil with no effect, (Shute.1939.), which was confirmed by others. (Haddow, Russell.1937.).

So far all the experiments tend to indicate that lack of Vitamin E results in uncontrolled proliferation of certain cells, possibly resulting in definite malignant changes. Why this should occur is uncertain. There is no doubt that lack of the vitamin disturbs the function of the nuclei and this may be sufficient to cause it.

On the other hand as has been mentioned before the vitamin is necessary for the proper division of certain cells, such as those of the central nervous system, possibly being essential for rapid mitotic division or for the production of important cellular components like Nissl's substance, which is hard to fit in with the above results. Also it has been said that

Vitamin E may play a part in the general body growth by a stimulating effect on all cells, a very much smaller quantity being needed for this function than is needed to prevent changes in the growth of the foetus or of the testes. (Mason, 1933a.). Finally Vitamin E is closely allied to certain Carcinogenic Compounds as well as to Cholesterol, Calciferol, Androgens, Progesterone and Oestrone, which are all derivatives of choline, and it has been suggested that it might play a part in the metabolism of this anthracene group. (Adamstone, 1941g).

This general stimulating action and the similarity of Vitamin E to ~~other~~ choline derivatives is more in favour of an excess of the vitamin, rather than a lack, producing malignant changes, but further experiments will be needed to clear up this question. Certain Authors have noted carcinomatous tumours from feeding rats on crude wheat germ oil. It was ether-extracted, washed with ½ the volume of 4 per cent Na OH and concentrated by distillation. 1.c.c was given daily for a hundred and sixteen days and rapidly growing abdominal tumours noted. The malignant nature of these was proved by this rapid growth, and by the fatal outcome, by their implantation through six generations and by the morphology of the cells which resembled a spindle-cell sarcoma. All the tumours remained intra-abdominal and none penetrated the external musculature or cutaneous tissues. They sometimes produced intestinal obstruction or invaded the ovaries, kidneys, liver, spleen, diaphragm and lungs. None of the animals recovered, and it was possible to transplant the tumours from one rat to another but not to rabbits, guinea-pigs or mice.

These experiments are of little value as although different strains of rats in other laboratories showed similar tumours, mice and guinea pigs under the same treatment were unharmed, showing it was species specific and the tumours were only produced by the sediment of the oil and not by the supernatant fluid from the top of the bottle. ~~Also~~ It is almost certain they were due to some other factor than vitamin E in the oil, as wheat germ itself, refined ether-extracted oil, gasoline-extracted oil, compressed wheat germ oil and Vitamin E concentrates failed to cause them; and the tumour-producing agent could be destroyed without affecting the Vitamin E content. The carcinogenic agent is probably inherent in the germ or produced during the process of extraction. (Rowntree, Steinberg, Dorrance, Ciccone. 1937.).(Rowntree, Landsbury, Steinberg, 1937.). The addition of milk, vitamins, calcium and iron to the diet had no effect on their incidence (Ross, Perlzweig, Taylor. 1938).

Other Authors have failed to produce tumours in rats with wheat germ oil. (Evans, Emerson. 1939.).(Dingmouse, van Ecke. 1939.).

To close this section the relationship of Vitamin E to the haemopaetic system will be discussed. Dogs

deficient in Vitamin B develop a hypochomic anaemia ~~anaemia~~ unrelieved by iron, and patients suffering from Pellagra and Pernicious Anaemia or simple Pernicious Anaemia show a five per cent reticulosis and increase in the number of white blood cells on administration of the same vitamin either orally or parentally. It does not act specifically or as true anti-pernicious anaemia factor, (Viltro, Shirer, Spies. 1940). Now it is stated that Vitamins B and E may in some way be related to the production of certain diseases of the nervous system, so they might be similarly so in haemotological diseases. There is little evidence to support this.

Vitamin E has been stated to increase the erthyocyte count and haemoglobin concentration in dogs. (Pergallo, Fieri. 1938.). Lack of Vitamin E in chicks produces a condition not unlike Leukaemia although it is more similar to a lymphosarcoma. (Adamstone. 1936.). This has been described in detail previously in this section. Adamstone also noticed a condition of anaemia in newly hatched chicks fed on rations treated with ferric chloride and halibut-liver-oil, the former being used to destroy the Vitamin E content. They showed a loss of colour in the shanks, being almost pale gray at times. The liver was swollen and showed dark mahogany-brown spots on its surface. The marrow of the long bones was firm and dark red. There was no change in the spleen. On examination of the lesions of the liver, the sinusoids were found to be widened, the liver cells enlarged, and the monocytes and Kupffer cells unusually numerous. The increase in the phagocytes is accompanied by extensive destruction of the red blood capsules, with deposition of the iron compound, haemosiderin, as granules in the liver cells. There is an increase of Myeloid tissue in the bones to compensate for this destruction. Its cause is uncertain It may be due to the destruction of Vitamin E or some other substance, essential to the red blood corpuscles, in the diet; Or this unknown factor may be absent from halibut liver oil but not from cod liver oil or sardine liver oil, as in a previous experiment similar changes were not noted when these last two substances were used in place of the halibut liver oil. The destruction of Vitamin E is apparently a necessary factor in producing the condition as a normal diet with halibut-liver-oil added produces no effect. On the other hand it cannot be prevented by giving Vitamin E alone, with a similar diet and oil, as used in ~~the first~~ this experiment. The damage is confined to the liver oil and red blood corpuscles, the damage to the former being secondary to destruction of the latter. (Adamstone. 1941.b).

However, Shute found no improvement in children with leukaemia on treatment with Vitamin E, (Shute. 1939.), and Evans demonstrated that there was no change in the peripheral blood in Vitamin E deficient experimental animals, even though the corpuscles of the blood are derived from areas of rapidly proliferating cells. (Evans. 1932.). Cases of malnutrition with an associated macrocytic anaemia have been stated to show a slight reticulosis in the first week after injections of Vitamin E, but showed no increase in the concentration haemoglobin or in the

red blood count. Pernicious anaemias in relapse with postero-lateral degeneration of the spinal cord and peripheral neuritis also showed no improvement or alteration in the blood picture after injections of five hundred milligrams of α tocopherol. (Spies, Viltner. 1940.). Other Authors state that they found there was no anaemia in Vitamin E deficient experimental animals. (Hegan, Hardshaw, 1926.), (Sure. 1929.).

Vitamin E was at one time thought to have a rôle in the assimilation of iron (McCallum, Simmonds, Becker. 1925.). This was later discovered to be due to destruction of Vitamin A by ferrous sulphate which was used in the original experiments, and in states of Vitamin E deficiency the iron metabolism was found to be unaltered and the erythrocyte count did not differ from normal. (Simmonds, Becker, McCallum. 1928.), (Sure, Kik, Walker. 1929.).

So, even although haemopaetic tissue is capable of tremendous adjustments, which might not reveal in the blood extreme changes occuring at an early level in maturation of the cells, the weight of evidence is that the haemopaetic function is unaltered by lack of Vitamin E.

For the sake of completeness it may be noted that, on the grounds that Vitamin E is a general stimulant to cell growth, it has been tried in conditions such as superifical wounds where healing is taking place, with reported benefit. (Leranth, Laszlo. 1936.). Skin wounds in rats on a diet rich in Vitamin E healed faster than normal, (Pegreffi. 1935.), and in the skin of rats on a Vitamin E deficient diet the papillae are reduced in size, the follicles are shallower, the glands smaller and the hair thinner and more pliable. These later changes are cured both by Anterior Pituitary than normal. extracts and by Vitamin E which shows there may be a link between these two substances. (Venzar, Kodas. 1931.). It has also been stated that the vitamin stimulates bone regeneration and callus formation. Although this is denied by some authors, others do suggest that its lack results in a slowing down of the processes of bony regeneration in fractures and in an inhibition of growth in adolescence. It is suggested that the Vitamin E content of the diet should be controlled in all cases in which there is slowness or even complete lack of bony regeneration and growth. Oestrogens and testesterone have also been used to stimulate bony growth. (Bartolomucci. 1940.).

B. The Effect of Vitamin E Deficiency on the Female Reproductive System.

(a) In Experimental Animals.

In this section only the results of Vitamin E deficiency on the reproductive system of experimental animals will be noted and the possible explanation of these changes will be discussed in a later section.

Pregnancy.

The attention of the early Workers in this field was

was drawn to the possibility of their being such a substance, as was afterwards called Vitamin E, by the fact that on certain diets female rats were unable to complete their pregnancies and rear normal young. For instance it was noted that if milk was the sole source of protein and vitamins the rats aborted after normal implatation and lactation failed. The ovaries were apparently normal, although the testes degenerated in males on a similar diet; but in spite of this the impairment of reproduction was greater in the female than the male, and even after returning to a normal diet reproductive ability was not restored for a long time. (Mattill, Stone.1923), (Sure.1924). The missing factor which caused these changes was at first thought to be Vitamin B1 but was then found to be a so far unknown factor, and it was called Vitamin E. These findings were confirmed by many Authors. (Evans.Bishop.1922), (Sure.1924), Hogan.Hardshaw.1926), (Suzuki.1927), (Juhász-Shaffer.1933a).

In rats the vitamin was apparently not needed for ovulation, oestrus, mating or implation of the fertilised ovum as these occured normally. The presence of red blood corpuscles on the thirteenth day of gestation being taken as a sign that implantation had occured. (Vøgt-Möller.1939). However, after the implantation of the blastodermic vesicle on the fifth day after copulation the absence of the vitamin begins to make itself felt. The pathological changes are almost solely confined to the embryo which develops slowly for even as long as the thirteenth day of pregnancy, but such findings as the high incidence of spontaneous deciduomata show that all is not well with the maternal tissues either. Details of these changes will be given below. (Evans.1932).

If the lack of Vitamin E is complete the pregnancy in female rats always ends in abortion or resorption of the dead foetus after about two weeks of pregnancy have passed, and sterility results till the supply of the vitamin is renewed. (Juhász-Shaffer.1931c, 1931d), (Sure.1926), (Evans,Burr.1925,1927). The term resorption was coined by Evans, and indicates that the dead embryo is disintegrated and removed by the phagocytic agents of the mother. On the other hand if the deficiency is a partial one the rat may produce a litter, which is apparently normal at birth, but the sucklings do not develop and die in infancy, being abnormal and stunted in growth by the fifteenth day.(Barrie.1937), (Clarke.1940). As the next pregnancy almost always ends in abortion, this phenomenon has been termed 'first litter fertility'. The Gestation may be rescued and proceed normally to term if the supply of Vitamin E is renewed before the fifth day after conception, but if it is lacking from the fifth to the twentieth day, pathological changes always occur. (Evans, Burr.1927). Thus in the rat it is seen that the Vitamin is especially needed for the developing embryo and less so far the maternal tissues. It may be noted that lack of Vitamin A affects mainly the maternal tissues of the female rat, resulting in foetal death, prolonged gestation and difficult parturition.(Mason.1935).

Whether it is equally necessary for the normal functioning of the reproductive system of other animals is difficult to establish. It seems to be necessary for mice, (Wilbur.1938.), (Brown.1939.), and for the hatchability and viability of young chicks.(Adamstone.1931.). The chick embryo deprived of Vitamin E dies within a week, although it is difficult to see how natural chicken food can be deficient in it. It may depend on faulty metabolism or absorption of the vitamin by the mother.(Cond.1929.), (Barnum.1935.). From studies in veterinary medicine it seems to be needed by cows. (Vøgt-Möller, Barry.1931a., 1931b., 1934.). Vøgt Möller noted that sterility in these animals was due to feeding rather than to pathological changes in the ovaries and uterus. He also found that the non-infective type of abortion in cattle was commoner in the early summer than the winter and that individual animals showed variations in their power to assimilate various vitamins. He mentions that it is difficult to define abortion in domestic animals as they are adeciduate and the dead embryo is either absorbed or desicated. However he treated one thousand two hundred cows with ten to twenty millilitres of wheat germ oil with an apparent success of seventy five per cent. (Vøgt-Möller.1939.),(Anderson.1934.) . Sows show signs of Vitamin E deficiency and, in cases of sterility in these animals, a cure-rate of seventy per cent has been quoted. (Vøgt-Möller.1939.). A diet rich in the vitamin also seems to reduce the mortality of sucking pigs. (Auble, Hughes, Lienhart.1929.). Hogs likewise abort on a Vitamin E-deficient diet. (Ewing.1935.).

Goats apparently do not need the vitamin in their diets nor do sheep (Thomson, Cannon, McNutt, Underbjerg 1938) (Drummond 1939b) and there is some doubt if Human Beings do (Wilbur 1938) but this will be discussed later.

The Embryo.

It has been noted that the main pathological changes in the Vitamin E deficient rat occur in the developing embryo. Details of these as found by various authors will now be quoted.

In the absence of Vitamin E the embryo develops slowly till the eighth day and dies between the tenth and twentieth day of gestation, undergoing autolysis and resorption or abortion. Vitamin E given in the early stages allows the gestation to proceed normally which indicates that irreversible changes do not occur till after placentation is well established and at a period of embryonic development when rapid growth and differentiation of its organic systems is occuring. The most detailed description of these changes is given by Evans and Burr.

The first deviation from normal occurs on the eighth day when signs of retarded development appear especially in the mesoderm and in the form of the amniotic fold. There is also no ectodermal cavity formed by then. On the tenth day the blood islands are smaller and fewer in number than normal, the gland like folds in the yolk sac are less evident and there is rarefaction of the embryonic connective tissue. The eleventh day shows a few blood islands

containing erythroblasts with pyknotic nuclei, and the almost complete emptiness of the blood vessels of the yolk sac. The villi of the latter are also underdeveloped. By the twelfth day the liver is a solid mass of cells with hardly any sinusoids and is not functioning at all as a haemopoetic organ. The heart and blood vessels only contain a few erythrocytes. The liver and blood vessels increase in size by the next day, but some of the embryos are found to be dead with atrophy of the allantois and capillaries. Evans and Burr state that the vitamin is probably needed in some obscure way by all foetal tissue, but that the middle germ layer and in particular the haemopoietic organs, the allantois, and the mesenchyme of the head, are especially sensitive to its withdrawal. No constant changes are seen in other embryonic tissues except some evidence of autolysis.

The nutrition of the embryo from the ninth day, when the yolk sac appears, till the eleventh day depends on the villi of the latter so that early foetal death results from any abnormality of these structures. Then nutrition occurs more and more through the allantoic route, after the penetration of the trophoblast by the umbilical vessels; but there are fewer allantoic projections than normal and these show signs of degeneration. These changes again result in starvation and death of the embryo but at a later stage. It may also be mentioned that the defective blood formation may be an added factor in causing mortality early in foetal life, while atrophy of the capillaries in the labyrinth with resulting asphyxia may similarly be a factor during the later stages. The thirteenth day seems to be the critical one.

The foetal division of the placenta relatively outgrows the embryo but is not normal in structure. The changes in the allantoic projections have already been mentioned. The invasion of the decidua subplacentalis by the syncytium proceeds almost normally except that the zone is more loosely arranged and wider than normal and contains vascular islands. This is due to the failure of the labyrinth to grow at the expense of this region because of capillary degeneration, with the result that the maternal blood seeps into the area. For the same reason the labyrinth is absorbed very soon after the death of the embryo. (Evans.Burr.1927.).

Resorption of the dead foetus is usually complete by the twentieth day, (Vøgt-Möller.1939.), the tissue first undergoing coagulative necrosis. The changes described above are different from those occuring from deficiency of Vitamin A, or any other cause, in the uniform degree of the histiopathological injury ~~and~~ occuring in different foetal sites at any particular stage of gestation, and in the primary involvement of the foetus and foetal membranes rather than the maternal placenta and uterus. (Mason.1939a.).

~~formation~~ The fact that Vitamin E is necessary for the blood ~~supply~~ and the nutrition of the rat embryo was confirmed by

Mattill,(Mattill.1939.), and the failure of the mesodermal elements in the foetal placenta by Ufner and others. (Ufner.1931.),(Zagoni. Sindoni. 1934.).

It will be noted that resorption of the foetus could not be prevented by giving the vitamin after the fifth day of gestation, although the first pathological injury was not demonstrated till the eighth day. This indicates that a physiological disturbance must occur before cytological changes appear. (Mason.1933a.).

Details of the pathological changes occuring in the Vitamin E deficient chick embryo have already been described, and it has been noted that, apart from signs of maldevelopment, lesions indicating unrestricted cell division occur. (Adamstone.1931.). The latter are not noted in the rat. Death of the chick embryo results from loss of blood, starvation, asphyxiation or a general toxic state. These causes are secondary to a degeneration of the circulatory system, haemorrhagic lesions and the development of the lethal ring. This ring has been described. It gives rise to a toxic state by causing haemorrhages and stagnation of the circulation, as well as causing death from asphyxia. If death occurs before the ring is formed it is due to the insufficient coalescence of the blastodermic blood islands or the inadequate anastamosis of the blood channels already formed, and if the embryo survives it, death most frequently occurs from internal haemorrhage. (Adamstone.1931.).

It may also be noted that chick embryos from mothers on deficient diets show skeletal maldevelopment. There is shortness of the bones, especially of the tarso-metatarsi and the antero-posterior axis of the skull. The diet was deficient in Vitamin E and wheat germ oil reduced the incidence of these findings, while vitamins B and G did not; but it is not quite certain if Vitamin E is the missing factor. Anyhow the factor is present in wheat germ, liver and whey, and its activity is increased by allowing the birds access to the sunlight and the green range of the spectrum. These reports lend some foundation to the theory that Vitamin E deficiency is a causal factor in the production of congenital abnormalities in animals and man. (Byerly, Titus, Ellis, Landauer. 1935.). In connection with this question of normal foetal development it may be noted that the stores of Vitamin A in Vitamin E deficient rats are decreased, and the power of the liver to absorb and retain it is lessened. As a possible result of the Vitamin A deficiency dental abnormalities such as whitening of the teeth have been found. These changes are not absolutely specific for Vitamin A deficiency, as they may also occur with Vitamin E deficiency alone, but they may be a common result of the deficiency of both vitamins. It is suggested that Vitamin E may stabalise Vitamin A in solution in the body fats, as hydroquinones do in vitro. No similar changes changes have been found with protein and vitamin B deficiencies, so the supplies of Vitamin E should be reviewed in all cases of vitamin A deficiency. (Davis. Moore .1941.).

The Maternal Placenta.

Vitamin E does not seem necessary for the normal development of the maternal placenta in female rats. The Placenta may go on growing and the mother increase in weight for a few days after the death of the embryo. It is normally smaller than normal. The few changes which do occur are probably secondary, and take the form of an overgrowth of giant cells and the occupation of spaces, which should be filled with foetal capillaries, by maternal blood and overgrown syncytium. (Evans, Burr. 1927.), (Eddy, Daldorf. 1941.). The degeneration of the foetal placenta, especially of its vascular elements, prevents the establishment of the normal connections between the mother and foetus. (Mason. 1939a.). About twenty days after conception, and seven days after the death of the foetus, the placenta strophies and is rapidly absorbed. (Vøgt-Möller. 1939.).

Changes in Other Reproductive Organs.

It has been stated that the changes in the female reproductive system of Vitamin E deficient rats and other animals are reversible, but this is not strictly true. It has been found that implantation becomes more and more difficult after each abortion or resorption, and this is not to be wondered at after a study of the changes in the uteri of rats suffering from prolonged Vitamin E deficiency. They become brown, and often enlarged, even in virgins, due to pigmentation and degeneration of the muscle. Fibrosis and solitary fibroma are sometimes seen. (Barrie. 1939c.). The pigmentation is due to the deposition of small yellow granules, and is confined to the muscular layer. (Moore, Martin, Rajagopal. 1939.). It is not absolutely certain if these changes are due to Vitamin E deficiency, but exposure to diets deficient in fats or Vitamin A did not produce them. They cannot be cured by Vitamin E concentrates, or by restoration of a normal mixed diet. (Martin. Moore. 1936.). However Barrie states that improvement occurs with the increased circulation which is present during pregnancy, and also maintains that pregnancy is unaffected unless fibroids are present. In his experiments the incidence of fertile matings was less among Vitamin E deficient rats, but the cause of this was not due to a difference in the rate of fertility, but to the fact that half of the experimental animals either do not mate or do not form a vaginal plug. Vitamin E deficient rats with previous resorptions were found to require a higher dose than usual of the vitamin to ensure normal gestation. (Barrie. 1938d.). The pigmentary changes are stated to occur about the tenth day of pregnancy, when partial vitamin E deficiency is present, and to become more marked with the progress of gestation. (Viner. 1931.). Their presence has been confirmed by other Authors. (Bacharrach, Allehorne, Glynn. 1937.).

Confirmatory signs that Vitamin E deficient female rats are not functionally normal are the high incidence of spontaneous deciduomata, (Evans. 1928a.), and the occurrence of abnormalities of parturition associated with foetal death near term. (Barrie. 1938b.). The ovaries are apparently unaffected, (Mason. 1939a.), (Biddulph, Meyer. 1941.); but it may be mentioned that many of the animals showed degeneration of the tubules of the kidneys and lesions in the liver, while others suffered from bleeding from the nose and rectum. (Martin. Moore. 1936.). A possible explanation for all these changes is the hyper-activity of the anterior pituitary. (McQueen Williams. 1934.).

The Size of the Litter and Abnormalities in the Young.

It has been found that female rats, partially deficient in Vitamin E, show a decrease in the number of sucklings per litter. The average number is not significantly changed in the first litter, but the second shows a marked reduction. There is also an increased mortality in the second litter, which is more marked than at first appears, owing to the fact that normal rats kill some of their offsprings while experimental rats usually do not. The results of Barrie's experiments in this field may be quoted:

	Number of Young.	Still Born.	Weaned.
44 litters of Vitamin E Deficient animals.	229.	14.	24.
44 litters of Normal animals.	295.	0.	274.

So it can be seen that normal rats have an average of 6.7 sucklings per litter and no still births, while the animals deficient in the Vitamin only have an average of 5.2 sucklings and have .32 still births per litter. (Barrie.1937a.). It has also been noted that the size of the litters varies inversely with the number of previous gestation-absorptions. (Juhász-Shaffer.1931c.), (Bacharach.1938b.).

Of the few offsprings that are born to Vitamin E deficient mothers, few reach adult life if weaned by them, owing to the deficient supply of Vitamin E through the mammary glands.(Barrie.1939c.). Evans and Burr were the first to describe the changes which occured before death, and were chiefly confined to the neuro-muscular system. (Evans. Burr.1928.). When fourteen to eighteen days old the rats were grossly underweight and undersized . There was an excess of fat and the fur was sparse, soft and white. Then weakness was noted in the feet, and an inability to grasp a rod. Their eyes moved slowly and were dull, and their habits indicated a subnormal intelligence. Finally they could not spread their toes, dragged their feet and suffered from carpopedal spasms and intermittent convulsions just before death. These spasms and convulsions suggest a decreased blood calcium, but serum taken from the sterile adults, as it was not possible to get it from the sucklings, averaged 11.5 mg of Calcium per 100 ml; the normal being 12.6 mg per 100 ml.. Also the spasms were not relieved by subcutaneous injections of 1c.c of 10% Calcium Gluconate or by Vitamin D. (Barrie.1937a.). The Histological appearance of the neuromuscular system will be described later, but it may be noted that ossification is retarded and the skull is soft, cutting like paper. Similar bony changes are seen in hypophysectomised rats. (Mortimer, 1937.).

As a result of these findings it is obvious that the birth of living young is not proof of an adequate supply of Vitamin E, because, although a few sucklings may be born normally, a greater number may be absorbed before the onset of labour. Few normal rats give birth to less than five sucklings per litter, so that if female rats deficient in Vitamin E are given a concentrated preparation of it and

produce more than five offsprings the dose can be said to be adequate, but if less than five it is not so. This is borne out by the fact that small litters are seldom reared. It may be stated that if continuous bleeding follows placentation resorption will almost certainly occur. (Barrie.1938b.).

Prolongation of Gestation.

Apart from the abnormalities in the number and development of the offsprings of Vitamin E deficient rats noted above, it has been found that the period of their gestation is prolonged. Barrie confirmed this with female rats fed on a diet which contained sufficient Vitamin E to enable them to produce two litters at least, although the young always became abnormal and usually died within twenty three days. For purposes of control he divided the rats into five groups. The first received no Vitamin E and all the foetuses were resorbed. The second were severely deficient in it, and although they usually produced living litters, they could not rear them; while the third, with slightly more of the vitamin, always produced living litters and sometimes reared them. The fourth group were fed on a Vitamin E deficient diet, but were given it in the form of concentrates, and the fifth were fed on a normal diet. Both these groups reared normal litters. The normal range for the duration of pregnancy is twentyone to twenty three days, and he found that 67·5 of the third group fell within this time but only 31.82% of the secondgroup. His results may be tabulated as follows:

Groups.	Rats.	Length of Gestation.	Number Born.	Born Dead.	Reared.
2.	22.	32 - 23.	71.	43.	1.
3.	40.	25 - 19.	242.	24.	62.
4.	27.	25- 22.	17.	17.	119.

These figures definitely indicate that the length of gestation is related to the amount of Vitamin E in the diet, although its prolongation is not caused primarily by the deficiency of the vitamin but by the embryonic mortality due to this. On post mortem examination it was found that as well as a few undersized but apparently normal foetuses, there were several undergoing resorption, and this process delayed the development of the former and so prolonged gestation.(Barrie.1939b.). The presence of Vitamin A had no effect on the results of the experiments. (Barrie.1939c.). There may also be a link between these findings and a deficiency of the Anterior Pituitary secretions, but this will be discussed later.

Shute states that gestation is not prolonged in states of Vitamin E deficiency in the rat.(Shute.1938b, 1938c.).

Lactation.

The fact that the supply of Vitamin E from the mother rat to her offspring is almost entirely through the mammary gland has already been mentioned. The result of this is, that if the diet of the mother is deficient in the vitamin, the sucklings do not receive sufficient of it for their development and begin to show abnormalities about eighteen days after their birth.(Barrie.1937d.). The experiments performed by Barrie to prove this will be described. Female

rats, proved to be sterile, were mated and given small doses of the vitamin so that they produced litters but could not rear them. Then in some cases these litters were changed for normal ones born on the same day and in others the change was made on the third, fifth and sixth days respectively after birth. In every case the animals fed by the Vitamin E deficient mothers became abnormal, although the ones exchanged on the sixth day were not so badly affected as the others.

In a further experiment a Vitamin E deficient female rat was given a dose of Vitamin E concentrate but none in her diet. She gave birth to a litter of eight. Five died at birth, one soon after and two grew slowly and died on the sixteenth day. This mother was then given four normal sucklings, aged three days, and fed them for eight days. After this two of these were returned to a normal mother, and exchanged for three of the latter's offsprings aged four days. So the first rat was now suckling two rats aged eleven days and three aged four days. The former developed normally as did those returned to the normal mother, but the latter developed signs of Vitamin E deficiency, such as weakness of the legs, on the eighteenth day. Finally it was found that Vitamin E deficient rats ~~were~~, given a large dose of the Vitamin on mating, ~~and~~ could rear their own litter, but not a second one belonging to a normal rat. These experiments show that the milk of Vitamin E deficient rats is lacking in some substance essential for normal growth and development of the young, that the maternal supplies are soon exhausted, and that if the young are fed on normal milk for a few days they may get sufficient supplies of this factor to tide them through the rest of the suckling period. In view of Evans' experiments which showed that muscular weakness in suckling rats could be prevented by giving Vitamin E before they were fifteen days old, it may be safely presumed that this Vitamin is the missing factor. It is also evident that the young are normal at birth as, even though their own mothers are deficient in Vitamin E, they can be reared by normal rats. (Barrie 1937a, 1938c.).

It has been suggested by some authors that Vitamin E is actually needed for the adequate flow of the milk, because lactation was not secured when the diet was deficient in it, and that this inadequacy contributed to the death of the young. (Sure. 1928.). It is possible that vitamins A or B or some unidentified substance may have been the missing factor in these cases, as there seems insufficient evidence that lack of the antisterility factor from wheat germ oil was the cause. (Sure. 1930.).

Other Experimentors state that the total quantity of milk from Vitamin E deficient mothers cannot be subnormal to any degree, because their offsprings are fat and their stomachs full of milk at autopsy. (Evans. 1924.), (Sure. 1926.), (Barrie. 1937d.).

The Pituitary.

The Histological changes of the Anterior Pituitary Gland

described in cases of Vitamin E deficiency in female experimental animals may be described here because of the possible etiological relationship between the Pituitary and vitamin deficiency changes in the reproductive system. Different Experimentors disagree about these changes.

Barrie's description states that the acidophil cells are small and degenerate with no distinct granulations. Often the nucleus is pyknotic and the cytoplasm stains evenly with acid stains. They are most numerous in the region of the cleft. There is also some degeneration of the basophils, and some empty cells are seen which are probably mostly derived from the acidophils. These empty cells give the anterior lobe a spongy appearance. One of this animals showed changes of particular interest. She was partially deficient in Vitamin E, and at puberty mated with a normal male. Six matings took place but placentation never occured. The animal was youthful in appearance with silky fur. On autopsy the thyroid was hypoplastic and the ovaries showed no normal follicles. The pituitary was almost entirely undifferentiated, with a few small degenerate vacuolated acidophils and sparsely granulated basophils. There were many empty cells. The Pituitaries were examined by the Wolf and Cleveland or Severinghaus techniques. (Barrie.1937a.), (Underhill.1939.).

Other Authors found that the pituitaries from pregnant and non-pregnant vitamin-E deficient female rats were normal, (Nelson.1933b.), (Stein.1935.), and Muller found a similar result in Vitamin E deficient does.(Muller, Muller. 1937.).

Vitamin E is also stated to have an effect on the gonadotropic hormone content of the female anterior pituitary. From tests on immature hypophysectomised rats some Experimentors found a decrease in the amount of luteinising hormone in pituitaries from Vitamin E deficient female rats, and also that the ovaries of these animals responded normally to extracts of pregnant mares serum. (Drummond, Noble, Wright, 1934.) Rowlands and Singer found similar results but in his experiments he used immature female rabbits. The Pituitary extracts from rabbits deficient in the vitamin, whether pregnant or not, showed a decreased power to produce ovulation. This did not become more marked on prolonging the deficiency, but the pituitaries from cured animals were more potent than normal to produce it in oestrus rabbits. (Rowlands. and Singer. 1935.). Nelson discovered no alteration in the power of these glands to stimulate the immature rat ovary, and states that this is what one would expect as reproductive failure in these cases only concerns the inability to maintain full-term gestation.(Nelson.1933a, 1933.b.). Finally McQueen Williams implanted pituitaries from vitamin E deficient female rats into immature ones, with the result that the ovaries increased in weight, presumably due to increased gonadotropic potency of the pituitary extracts. (McQueen Williams.1934.).

It is suggested that the difference between the results of Rowlands and Singer and those of Nelson anyhow is due to the fact that in rats the response of the ovary depends firstly on the power of the pituitary extract

to cause follicular growth, while in the rabbit it depends on the presence of the luteinising substance and third gonadotropic principle, and Vitamin E deficiency may cause a decrease in the quantity of the latter only. In these experiments the usual criterion of the amount of hormone in the pituitary material was the number of immature rats in the group under test which had corpus lutei in their ovaries, and showed an increase in weight of this organ.

The Thyroid.

Changes in the thyroid observed in experimental vitamin E deficient animals may also be described here as the thyroid is like-wise linked up with the reproductive system.

Singer carried out experiments to see if there was a decreased thyrotropic activity in these animals. First he noted that normal rats showed variations in the histology of the thyroid, but it was normally relatively hyperplastic and rich in thyrotropic substance. The metabolic rate was correspondingly high. Then he removed the thyroids from sterile female rats which had been fed for twelve to eighteen months, since weaning, on a Vitamin E deficient diet. The histiological changes found were similar to those seen in case of pituitary deficiency, so before thyroidectomy was performed some of the animals were given injections of anterior pituitary substance and another group received iodine for ten days to four weeks. The picture seen in the thyroid was one of inactivity. The vesicles were full of non-vacuolated colloid which was usually dense and eosinophilic. In some cases the cubical cells were flattened, while the nucleus showed slightly staining cytoplasm with well defined chromatim granules. The vascularisation of the thyroid was poor, and pregnancy did not produce any significant change in these or normal glands.

The pituitary extracts had small, if any effect, on the histiological picture, although fifty milligrams did produce some decrease in the colloid content of the gland. This is a surprisingly small response if the state of the thyroid is solely due to pituitary failure, as has been suggested, and not to the primary effect of Vitamin E deficiency. Iodine caused no histiological change.

If a curative dose of Vitamin E was given the thyroids showed signs of increased activity. The glands become hyperplastic with reduced colloid and high cuboidal epithelium. Vascularization was increased and the appearance was generally normal.

So it is conceivable that Vitamin E deficiency has a direct effect on the secretory processes of the thyroid, or the histiological changes may be influenced by the disturbed function of the pituitary due to its lack. The former theory could be proved if it was shown that Vitamin E restored the normal function of the thyroid in Vitamin E deficient hypophysectomised rats. (Singer, 1936). In noting this relationship between the thyroid, pituitary and the reproductive system it may be stated that van Horn found that hypophyseal implants, from ovarectomised rats fed on thyroid, produced no abnormal changes;

and that Gonad-stimulating extracts on injection into hyperthyroid and normal rats caused an increase in ovarian growth in all cases. However, he was led to believe that hyperthyroidism led to increased gonad-stimulating power of the pituitary, so that in Vitamin E deficiency the hypothyroidism, which is usually found should be associated with a decreased power, which has been found in some cases. His explanation was that the increased metabolism led to elimination of oestrogenic substances, so that oestrus could not occur and the pituitary was released from the inhibitory control of these substances. It might be thought that in the hypothyroidism of Vitamin E deficiency, there would be an excess of the oestrogenic substances, which would agree with Shute's theory. However, in experimental animals there was no excess after thyrodectomy, although an irregular oestrus cycle was noted. (van Horn. 1933.).

A state of thyroid hypoplasia with increased fibrous tissue between the vesicles, in vitamin E deficient animals has been confirmed by other Authors. (Barrie, 1937a.), (Underhill, 1939.).

Apart from these histiological changes Barrie noted signs of cretinism in Vitamin E deficient rats. They failed to grow, the head was large with open fontanelles, the fur was sparse, the nose unturned and in one case the forepaws were abnormal with one foot larger than the other, which was clubbed. The animals moved slowly and showed subnormal intelligence. They died in seventeen to thirty days. The Thyroid glands were small and pink. (Barrie.1937b.).

The Thymus is unaltered in experimental animals fed on a diet deficient in Vitamin E.

The Adrenal Gland.

Changes in the adrenals of pregnant rats with Vitamin E deficiency have been noted. They took the form of medullary atrophy and cortical hypertrophy. (Blumenfeld.1934.). Others found no alterations from the normal. (Biddulph.Meyer.1941.).

Oestrogens.

One of the theories to explain the action of Vitamin E is that when it is deficient there is an excess of oestrogenic substances in the blood, and that these substances exert an anti-proteolytic action. This will be discussed later.

To demonstrate this theory Shute fed rats on a diet deficient in Vitamin E, and tested the serum with protein and trypsin solutions. He found that 93% of the animals showed the presence of a factor, which resisted the digestive action of trypsin, after four months had elapsed and, that when mated at this time, resorption of the foetuses or abortion always occured. The time of the first appearanne of this factor varied greatly.

The factor disappeared on the addition of Vitamin E to the diet, and a quantitative relationship between the dose of vitamin E and the time of this disappearance was established. Six drams of viteol, which is a connentrated preparation of the

vitamin, renders the blood serum digestable in twenty-four hours, and one dram a day will maintain it so. It may also be noted that shaking wheat germ oil with serum from these experimental animals in vitro renders it digestible, but unfortunately other oils such as maize oil and petroleum oil have a similar effect, so that it may be due to the shaking. (Shute. 1936). Eleven per cent of normal animals also show the presence of the factor in their serum. (Shute. 1938b.).

Most of Shute's studies were on human beings so details of these will be given in the next section.

Toxaemias in Pregnancy.

Some Experimentors found that a partial vitamin E deficiency in experimental animals was more dangerous than a total one. This was apparently due to prolongation of gestation with death of the foetus at a late stage, so that unless abortion occured a large amount of tissue had to be absorbed by the mother which might lead to a state of toxaemia. (Barrie. 1939c.).

None of the experimental animals showed signs of illness till after placentation, but the time of onset varied. The animals became listless, refused their food and lost weight. The hair was rough and in some cases there was bleeding from the mouth, vagina and rectum. He divided the animals up into five groups. The first group had been on a Vitamin E deficient diet for five months and were unable to produce any litters. The second group had been on a similar diet for a shorter time but were also unable to produce litters, and the third had been on the diet long enough to result in sterility, but had received small but inadequate doses of the vitamin at the start of pregnancy, which resulted in the birth of dead foetuses. The fourth group were treated in the same way, but received a larger dose of the vitamin, so that they produced living litters. The fifth group consisted of normal control rats. The incidence of toxic symptoms in these groups was 2.32., 1.77., 19.4., 2.15., and 0 percent respectively. From this it can be seen that the toxaemia is typically associated with partial deficiency.

On post-mortem examination of the Vitamin E deficient experimental animals it was found that there was little increase in the size of the uterus after implantation, but on about the nineteenth day it was found to be filled with occult blood. The placental sight could be clearly seen but there was no sign of the macerated foetus. If the animals received small doses of the vitamin, the development of the foetus proceeded further and the mother gained in weight considerably, and it was among these animals that toxic symptoms were most frequently seen. They normally died within three days of the appearance of these the liver was damaged, but the renal changes were most striking. The tubules contained albumin; and in most cases calcium salts were deposited in the periphery of the medulla, which was probably secondary to excretion of the albumin. Fat was also sometimes found in the tubule cells.

Minor degrees of these changes, which indicate kidney damage, were found in all cases where resorption had occured, even although no toxic symptoms arose.

It has already been mentioned that the toxaemia is probably

related to the absorption of the foetal material. It has been suggested that it is the calcium and phosphorous from the foetal skeleton which causes the damage, but it is difficult to see how this can be the case as the blood calcium in pregnant rats is always low, and toxic symptoms appear by the thirteenth day when the foetal skeleton is only just beginning to develop. However, whatever the factor is, it most probably arises from the foetus or placenta, and the toxic condition is not necessarily due directly to deficiency of Vitamin E. No experiments have been carried out to test the efficacy of Vitamin E in curing the toxaemia of these animals, but one would not expect this to take place once the condition was established, although it might prevent it. (Barrie.1939a.). In support of these findings it may be noted that German Authors reported somewhat similar kidney changes on injection of the products of Placental autolysis.(Shute.1939.). The role of the Pituitary in the etiology of this toxaemia has not been clarified.

Toxaemia of pregnancy and Similarity between these symptoms and the conditions of Abruptio Placentae in women has been noted,(Hume. Henderson-Smith.1938.), and it has been suggested that in Humans also a partial deficiency of Vitamin E is more dangerous than a total one. (Shute.1938c.).

Abortus Fever.

There is no doubt that abortus fever is due to the invasion of a micro-organism of such virulence that all the intrinsic powers of resistance of the animal are overcome. However as an adjuvant to this, the lowered resistance of the individual animal from malnutrition or excessive sexual strain may play a part, so Vitamin E has been tried in the treatment of this disease on the theory that it would increase the resistance to Bang's Baccillus, although it would not affect its virulence. (Lange.1938.). Favourable reports are given by some Authors,(Moussu.1935.), (Vøgt-Möller.1939.),unfavourable by others. (Risse.1937.).

(b).In Human Beings.

In this section the conditions in clinical medicine,which are thought to be possibly due to deficiency of Vitamin E, will be mentioned, and a few of them described in detail. The results of their treatment with vitamin E will not be given till later.

There is no evidence on general grounds that the vitamin is needed for normal pregnancy in women,(Browne.1939.), but the points of similarity between the conditions just described in experimental animals and certain diseases of women, the results of therapy, and the investigations of chemical workers, indicate presumptive evidence that it may be. (Bacharach.1940.). The fact that the vitamin appears to be linked up with the function of the endocrine as well as the reproductive systems in experimental animals has led to its trial in many and varied diseases.

Various explanations have been brought forward to explain why, on the same diet, whether it be a normal or deficient one, some people should show signs of deficiency while others do not. It is possibly due to a personal idiosyncrasy in the person's power to assimilate or utilize the vitamin, or to increased demands of the developing embryo. Some may inactivate the vitamin, and the theory has been suggested that lack of Vitamin Bi might lead to a 'conditioned' deficiency of vitamin E, so that a normal supply would not be sufficient. However vitamin Bi does not lower the effective dose of Vitamin E, or have any effect on such diseases as threatened Abortion. It is not present in crude wheat germ oil. (Bacharach.1939.). None of the cases treated developed malignant tumours which is against Vitamin E having a carcinogenic action.

These problems can only be cleared up by large scale investigations carried out with controls, and using pure preparations of the vitamin; as in using wheat germ oil the presence of other curative factors cannot be excluded.

Habitual Abortion.

The links between habitual abortion in female rats, deficient in Vitamin E, and the similar condition in human beings are that the general health is good, that mating and implantation are frequent and occur at short intervals, and that each successive pregnancy is blighted. (Vøgt-Möller. 1939.), (Juhász-Shaffer.1933.).

Many authors have reported success in the treatment of Habitual Abortion with Vitamin E, even after placentation is complete, (Vøgt-Möller. 1939.), (Macdonald .1939.), (Young .1937.), (Watson.Tew.1936.), (Currie.1935.), but equally good results seem to be recorded when using other therapeutic agents such as progesterone, vitamin C or pregnancy serum. (Krohn, Falls, Lackner. 1935.). Others doubt its value especially when abortions occur in rapid succession, owing to the permanent damage done to the uterus. Shute records three cases which aborted three or four times in fourteen months on large doses of Vitamin E. (Shute.1939.), (Bacharach. Allehorne, Glynn.1937.), (Browne.1939a.).

In spite of this the 75 percent success of such observers as Vøgt-Möller cannot be ignored. The difficulty is assessing the value of the treatment is in not knowing how many would have had normal pregnancies without vitamin E. For instance, Browne records eighteen cases of habitual abortion, eight of whom received progesterone, three vitamin E, and seven no treatment at all, and all of the last group produced living children. (Browne.1939a.). Other problems which compel a conservative outlook on this subject are the lack of exact knowledge of the cause of habitual abortion, and of the factors which contribute to the normal progression of pregnancy.

Shute admits that the 75 percent successes reported by Vøgt-Möller, the 72 percent by Watson and Tew and the 86 percent by Currie are not as high as might be hoped, but states that the failures may be due to personal idiosyncrasies on the

part of the patients. They may be unable to assimilate the vitamin or suffer from hypothyroidism. There may also be seasonal depletion of the vitamin, or the oil may be kept at too high a temperature. The cases not accounted for in this way are perhaps due to other causes such as infection, trauma, or abnormalities of the spermatozoa; although even these may have a background of vitamin E deficiency. In support of these theories he cites eight of his own failures; two of them showed no antiproleolytic factor in their serum, which he takes as a criterion of vitamin E deficiency, two of them were taking wheat germ oil which was rancid, one took a green diet instead of the oil for a time, and one took no oil for the three days prior to labour. (Shute. 1938b).

The variations in the results of various Authors may be due to the fact they they do not all adhere to the strict diagnosis of this condition, which may be defined as premature delivery on more than one occasion occuring before the sixth month of pregnancy. There must also be no discoverable disease or organic cause, nor must it be artificially induced. Its incidence among all pregnancies has been stated to be .41 percent by Bishop and 1 percent by Malpas. (Bishop. 1937.), (Malpas. 1938.). It may be also noted here that American Authors have found that 78 percent of all pregnancies end in abortion, but that 65 percent of these were artificially induced.

Finally Shapir contends that it is better to ascribe the beneficial effects of Vitamin E in habitual abortion to an excess, rather than to a correction of a deficiency. (Saphir. 1936.).

Chances of Full-Term Pregnancy in Untreated Cases of Habitual Abortio

It has already been mentioned that if the chances of pregnancy going to term without treatment in cases of Habitual Abortion are not known, it is difficult to assess the value of a therapeutic agent in the treatment of these cases. Malpas was the first to try and elucidate this problem. He states that eighteen percent of all pregnancies end in abortion and other Authors agree with this figure. (Whitehouse. 1929.) He derived it from the study of two thousand cases, and found that most only aborted on one occasion, proving that the most common causes of abortion are non-recurrent ones such as trauma, infection, faults in the implantation site and transient metabolic or endocrine disturbances. He also found an appreciable number of cases of sequential abortion due to the chance incidence, in a succession of pregnancies, of accidental or random causes.

Then he set out to discover how many abortions a woman must have before the presence of a truly recurrent cause can be reasonably inferred. He found that after three or more abortions random causes produced a negligible number of further cases, so that women who abort this number of times could be called habitual abortors. He based this hypothesis on the fact that the percentage of 1st, 2nd, 3rd and 4th pregnancies ending in abortion were 18, 4, 1 and 1 percent respectively, and stated that when the percentage no longer varied all cases must be due to recurrent causes.

If the recurrent factor was large, random causes

produced a negligible number of cases ~~even~~ after the second abortion.

In the Authors series of cases only one percent had three or more abortions so that random causes must account for seventeen percent of cases.

The number of cases which would go to term without treatment, or the spontaneous cure rate, is linked up with the value of the recurrent factor. It is high if the latter is low and vice versa. So if the Author's findings are correct it is high. After four abortions he states that the spontaneous cure rate is six percent, after three twenty seven percent, after two, sixty two percent and after one seventyeight percent.

The process of the normal development of pregnancy is essentially a continuous readjustment of the environment to the changing needs of the foetus, so the recurrent factor is probably made up of several causes such as lack of vitamins, hormones, minerals or essential amino acids like lysin and arginin. Malpas does not think that lack of Vitamin E plays a large part in causing Habitual Abortion as he only reports two successes on treating seven cases with the vitamin, and he also criticises the high claims of some Authors for any specific therapy. Even if the recurrent causes are removed by special treatment the patients are still subject to random causes influencing seventeen percent of all pregnancies, so that no results can be better than eighty three percent.(Malpas.1938.).

To apply these results to the reports of the effectiveness of Vitamin E in the treatment of Habitual Abortion, it may be noted that Watson and Tew treated seventeen patients, who had had two previous abortions, with Vitamin E, and had five failures. This was a cure rate of seventy percent, while the spontaneous cure rate of this group is sixtytwo percent; so the margin of cures presumably due to Vitamin E is small, but it is larger in other Authors' groups. These and other results may be tabulated:-

Percentage of Cases going to term.

Number of Previous Abortions.	Vøgt - Möller. 1.	Vøgt - Möller. 2.	Watson.Tew.	Total.	Spontaneous Cure Rate.
Four or more.	100	72	86	79	6
Three or more.	89	70	71	73	27
Two or more.	92	73	76	75	62

From these figures it does seem the Vitamin E has a direct effect in making full term births possible in cases of Habitual Abortion, and this may be even truer in the light of criticisms of Malpas's findings.

He ignored that the expectation of pregnancy decreases with each successive pregnancy. Taking this in account it is found that random causes operate in sixteen percent of all cases of Habitual Abortion and recurrent causes in two percent. Also the percentage of abortions does not really become constant after the third pregnancy but decreases indefinitely. Some Authors state that the chance of the next

pregnancy ending in abortion is as high as seventy percent after the first and thirty percent after the second. The agreement between theoretical calculations and clinical observations is better after these amendments have been made.

Finally it may be said that on an average the cure rate of cases of Habitual Abortions treated with Vitamin E is seventy five percent, which is higher than would be expected without treatment, but some of the patients treated had had only one abortion so were not habitual cases.

Threatened Abortion.

Threatened Abortion has been successfully treated with Vitamin E, and so the lack of the vitamin may be a causative factor in this condition. (Vøgt-Möller. 1939.), (Watson. Tew. 1935.), (Shute. 1939.), (Currie. 1937.).

Others did not report so favourably, although admitting that in some cases treatment may have been started after the abortion was inevitable or the foetus dead. (MacDonald. 1939.).

Equally good results have been recorded with treatment by progesterone, or merely by rest and the avoidance of violent exercise, marital relationships and drastic purges. (Browne. 1939a.). The suggested connection between Vitamin E and the Corpus Lutein may explain the similarity of results of treatment with the vitamin and with progesterone (Browne. 1939b.). No investigations with controls have been carried out, and this, and the necessity of having a common definition of threatened abortion may be stressed before assessing the value of any remedy. Threatened abortion occurs when there is bleeding, but no part of the ovum escapes or protrudes through the internal os.

It has been suggested that Vitamin E stimulates the production of the Luteinising Hormone by the Pituitary, which raises the question if it is safe to give Vitamin E early in pregnancy without excluding the presence of a Vesicular Mole, as it might foster and aggravate this condition. (MacDonald. 1939.).

The fact that in many cases the symptoms of threatened Abortion disappear spontaneously makes it difficult to assess the value of any specific treatment.

Sterility.

Vitamin E seems to play no part in the treatment of Sterility, although this does not mean it is not a causal factor in the female. It may be that a irreversible change takes place. The fact that some Authors report that Vitamin E has no oestrogenic or gonatotropic properties are against it having anti-sterility properties. (Saphir. 1938.). The reports of treatment are negative. (Vøgt-Möller. 1933b.), (Watson. Tew. 1936a.).

It must also be taken into account that if the husband is on a similar diet to his wife his spermatozoa may be morphologically defective and so cause sterility.

Toxaemia of Pregnancy and Abruptio Placentae.

It has been noticed that sometimes the same factors which are responsible for abortion are responsible for accidential haemorrhage and late pregnancy toxaemia. The women appeared to have resident in their bodies some morbid influence, not inconsistant with good health between pregnancy, but inconsistent with the normal continuation of pregnancy to term. (Young.1927.). Some of the damaged pregnancies ended in abortion, some in premature birth or stillbirth, and a few in a state of toxaemia. The latter only occured in the last few months of pregnancy, and a somewhat similar state occuring in experimental animals has already been mentioned. It was first suggested that the missing factor causing these toxic changes was progesterone, and success was recorded with its utilisation in their treatment. (Johnstone, Weisner, Marshall.1932.), (Krohn, Falls, Lackner.1935.), (Kane.1936.), and A similar success was found after using urinary prolans and Vitamins A and E.

In spite of this, experimental evidence is in favour of the lack of Vitamin E being the morbid influence in these toxaemic states; although Young gave both Prolan, progesterone and Vitamin E to patients suffering from them with no result, except that in a few instances of hypertension the blood pressure was lowered and the headache and visual disturbances went under the influence of Prolan. (Young.1937.). However, Shute found that if Vitamin E was given early enough at the first rise of blood pressure, increase in weight or appearance of oedema, these symptoms were abolished, and some of the foetuses gained in size. Even when the toxaemia had resulted in renal damage he reported that the systolic pressure fell under Vitamin E treatment, and the pregnancy proceeded normally. (Shute.1937a.). Macdonald could not confirm these results and does not think that abortion and toxaemia of pregnancy result from the same cause. (Macdonald.1939.). The success reported with the use of progesterone in these cases may be explained by the theory that vitamin E has a specific significance in the nutrition of the placenta and operates through the activation of the luteinising mechanism.

It may be mentioned here, that in the later months of pregnancy the placenta takes over the production of the leutenising hormone and progesterone, so that if it was damaged by lack of the vitamin, the giving of all three substances might be expected to improve morbid conditions resulting from this damage. In conditions of hydatidiform mole and chorion-epithelioma it is the foetal placenta which contains most of the luteinising hormone, while the maternal placenta and tissue of the ovary contain most of the progesterone. This suggests the place of origin of the two substances may be different, and that Vitamin E may have a special and differential relationship in regard to the tissues concerned. (Young.1937.).

It has been found in similar conditions in experimental animals that, for success, the treatment must be started before placentation occurs; so that in humans the causal factor has been operating for some months before treatment is started,

although no symptoms have occured till late in pregnancy. Henry states that often a rising blood pressure, as evidence of toxaemia, may be discovered as early as the fourth month of pregnancy. (Henry. 1936.). These facts may account for the failure of the vitamin in treatment reported by some Authors. It may be suggested that all women with histories of repeated abortion, receive treatment from the start of pregnancy to counteract this risk of toxaemia occuring in the last trimester, and that special care be taken with these cases who have recovered from a threatened abortion, as sufficient damage may remain to initiate toxaemia at a later date.

Where there is an unbroken sequence of Habitual Abortions, Stillbirths and Toxaemic symptoms, the breaking of this chain with the birth of a live child after treatment with Vitamin E, is one of the most suggestive findings that these conditions are linked with a common cause, and that, in states of partial Vitamin E deficiency, although there may be sufficient to prevent abortion, the pregnancy is still open to risks of toxaemia at a later date.

Allied to these states of toxaemia is the premature separation of a normally implanted placenta, which is practically always followed by symptoms of toxaemia. It is known as accidental haemorrhage, concealed haemorrhage, ablatio placentae and abruptio placentae. The last term is the best. Shute records the successful treatment of this condition with Vitamin E, even when it occurs in the later months of pregnancy.

The pathology of abruptio placentae is a rupture of the placenta from its site, and a tearing of the vascular elements of the decidua basalis with extravasation of blood into the uterine wall, amniotic sac or vagina. A somewhat similar picture has been described in Vitamin E deficient animals, but the placental architecture of these does not quite correspond to the human one. Attempts were made to cause premature separation of the placenta in rabbits by administering oxylates and uranium and producing a nephritis, and with a superimposed Bacillus Pyogenous infection in addition, (Brown, Dodds. 1938.), and subplacental haemorrhages were produced in experimental animals by intoxication with histamine, and by the injection of oestrin or anterior pituitary preparations. (Hofbauer. 1926.), (Zondec. 1929.), (Kelly. 1937.), (Tell. 1936.). However none of these conditions corresponded so closely as that produced by Vitamin E deficiency in experimental animals to abruptio placentae. (Vogt. 1934.), (Evans. 1932.).

Shute records a group of signs and symptoms occuring in women which may at any time come to be associated with uterine haemorrhage and interruption of pregnancy, and recommends that Vitamin E be given immediately these occur. First there is a gradual appearance of a restricted area of uterine tenderness with a continuous sacral backache. Occasionally uterine contractions, violent foetal movements, and uterine haemorrhages of any grade, appear. Then there is a gradual elevation of the blood pressure, oedema of the extremities, an increase in weight, and even albuminuria. This last is the usual evidence of the onset of toxaemia of pregnancy. In some instances attention is focussed on the case because

the foetus is unusually small, or there is an excess of amniotic fluid.

The uterine tenderness has been found on operation to correspond to the placental site, and it may disappear spontaneously or under treatment with Vitamin E, but after it has occured the placentae always show pathological lesions. It must correspond to a premature placental separation of varying degrees of severity.

Shute found no lack of Vitamin C in these cases, but he did find an excess of his antiproteolytic factor in the blood serum even before the occurence of symptoms. He associates the presence of this factor with lack of Vitamin E and his experimental evidence for this will be discussed in the next section. In fact he found the correlation between the onset of the clinico-pathological syndrome discussed above, and the occurence of this factor in the blood serum, was as clear as with cases of abortion and miscarriage. So directly the symptoms appeared he gave Vitamin E, and his reports are encouraging. He suggested that the therapy is of little use when complete separation of the placenta has occured; so maintains that a close watch could be kept for uterine tenderness, and that the blood serum should be tested for the presence of this factor early in pregnancy. If this is done the incidence of abruptio placentae may be reduced to small dimensions. (Shute. 1937a.). In cases of late toxaemia it is worth while giving vitamin E, as although they may not improve clinically, the placentae may adhere well enough to enable induction of labour to proceed normally.

Finally it may be mentioned that Shute divides cases of pregnancy toxaemia into those which go on to eclampsia, and those which result in abruptio placentae. He found only one out of a series of eight cases of eclampsia showed signs of Vitamin E deficiency as judged by the presence of his antiproteolytic factor, and in some of these cases it was reduced below normal levels. As his factor was of an oestrogenic nature he treated cases of eclampsia with oestrogens and reported several cures, and noted that wheat germ oil given to similar cases might initiate convulsions. He also found that the cases of eclampsia often showed an excess of Prolan in their serum. (Shute. 1937b, 1937c.). This was confirmed by other Observers. (Smith, Smith. 1935.). It has also been found that cases of premature separation of the placenta show a large urinary secretion of oestrin, (Hein. 1934.), and eclampsias of prolan, although sometimes also of oestrin.

Cases of eclampsia were often hyperthyroid, (Hoffmann, Anselmino. 1931.), while the ones suffering from abruptio placentae were hypothyroid, which fits in with the theory that Vitamin E deficiency is more common when there is lack of thyroid secretion. Other Authors have noted that abruptio placentae is rarely associated with eclampsia. (Holmes. 1923.), (Baird. 1935.), (Davis, McGee. 1931.).

However Theobald states that the two conditions are similar and are caused by absorption of toxins from the intestinal canal, which, owing to the breakdown of the defenses of the body, are not detoxicated. The cause of this breakdown may be a deficiency of ionised calcium in the blood. He denies they are caused by a placental toxin, or an organic lesion in the kidney. (Theobald. 1930).

From these findings it appears that abruptio placentae is as likely to be caused by lack of Vitamin E as by trauma, endometritis, diseases of the ovum, emotional states and toxaemias. The mode of action of the Vitamin E deficiency in these cases will be discussed when the various theories, to explain how its deficiency results in any pathological change, are reviewed.

Shute never recorded a case of placenta praevia which showed an excess of his factor in the serum, so this may be a valuable diagnostic aid to differentiate between it and accidental haemorrhage.

The Antiproleolytic Factor in Vitamin E Deficiency.

It has already been stated that in conditions of Vitamin E deficiency, Shute found that the serum of experimental animals contained a factor, which was antagonistic to the action of trypsin, and was oestrogenic in nature. He found a similar factor in Human Beings in conditions which were supposed to be due to a deficiency of the same vitamin. Many of his original experiments were carried out on Human Beings so these will now be described. He admits that abortion may be due to a variety of causes such as defects of the spermatozoa and ova, heredity, placentitis, congenital malformations, poisons, dietary defects, trauma, endocrine disturbances, placental sclerosis, changes of the ~~nervous~~ venous pressure and carbohydrate metabolism upsets. However, he considers that lack of Vitamins, especially Vitamin E, is one of the most important causes.

On implantation the trophoblast burrows into the endometrium, and Shute suggests that abortion occurs when the environment of the trophoblast is unsuitable, resulting in an increased maternal resistance to such an invasion. This has also been suggested by other Authors, (Blair, Bell. 1929.), (Grafenberg. 1909.), (Mall. 1917.), and a decreased lytic activity of the trophoblast has been observed in aborting rats. (Zagami. 1935.).

So faulty implantation of the ovum would be due to a maternal rather than a foetal defect, and this increased resistance to the invasion of the trophoblast has been found to be due to abnormal anti-proteolytic powers of the maternal blood serum. (Flexner, Berkson, Winters, Wolman. 1929.), (Grafenberg. 1909.). So Shute decided to find out if these powers were increased in cases of Vitamin E deficiency. The proteolytic ferment of the trophoblast resembles trypsin, so this was used to test the anti-proteolytic properties of the serum from supposedly Vitamin E deficient women. The blood was normally tested one to twenty three days after the start of an abortion, and control samples of blood were taken from

normal women who had had normal pregnancies, tryspin being added to both. Two sets of tubes were used and one of these was heated. Then the acid products of digestion were titrated against N/100 Sodium Hydroxide, using phenalphthalein as an indication, and it was found that in the cases of the serum from abnormal pregnancies the digestion was delayed for as much as ninety minutes. This delay might occur in the heated or the unheated set of tubes or in both. The resistance, the digestion might appear a months or more before the abortion occurred, and disappeared about six days after it had occured. If the uterus was curetted it was absent after two days.

It is obvious that this resistance might cause abortion, as in vivo fresh serum is coming to the placenta all the time. The causative factor is not the anti-~~typhoid~~ tryptic of

the blood as it behaves differently to heat and other agents, in fact this factor works better at higher temperatures, which may account for the frequency of abortions during fever. (Shute, 1938a.).

The serum from cases of abortion in women could be heated to 90° Centigrade for thirty minutes, and still show this resistance to digestion, but it was rapidly lost on standing or shaking, especially at room temperature. This is not prevented by such preservatives as toluol or phenol. Its disappearance soon after abortion suggested a relationship between the integrity of the placenta and the presence of the factor in the blood serum, and raises the possibility that the placenta excreted it. So Shute extracted placentae at every stage of gestation, and also the curettings of spontaneous and induced abortions. The extracts were added to normal serum, and tested for their resistance to proteolysis, with positive results in some cases. Ordinary blood serum of patients was used as a control, to show the action was not due to the blood in the placenta. Heating had no effect on the extracts showing antiproteolytic activity, but shaking with chloroform destroyed it.

Preparations of oestrin added to normal serum also gave it a similar antiproteolytic power. These findings indicate that this antiproteolytic property of certain sera might be derived from the placenta, as it contains oestrogenic substances which reproduce these same effects. These substances are more easily recovered from the placenta early in pregnancy, especially in cases of spontaneous abortion and miscarriage, as in these cases they are present in excess. They resist heat, are soluble in water and chloroform, oxidise fairly readily in an aqueous solution, disappear from the villi when they necrose and are present in the placenta throughout gestation. It cannot be prolan or antitrypsin, as these are destroyed by heat and, as it has been found that oestrin has the same antiproteolytic powers, it is probably actually oestrin or some closely allied substance. The placenta produces or acts as a reservoir for oestrin and, although the factor is more soluble in blood serum in vitro than oestrin or oestriol, oestrin in the tissues may be in a very soluble form.

In support of these theories, it may be noted that five to ten per cent of normal serum added to antiproteolytic serum renders its digestion in vitro normal, and cases of abortion have been treated successfully with small amounts of normal serum. Shute concluded from these experiments that an oestrogenic substance with antiproteolytic properties, and vitamin E, exist in a kind of equilibrium during pregnancy, and if there is an excess of the former, abortion results. (Shute, 1935b.).

excess
He records this in seventy three per cent of cases of spontaneous abortion (Shute, 1936), and suggests that the testing for its presence in the serum takes a place in the management of hyperemesis gravidarum, menorrhagia, many post-menopausal complaints, and in the diagnosis of acute appendicitis from placenta praevia and premature placental separation. Many women past the menopause excrete oestrogens in their urine, which shows that oestrogens still play a part in the body function at that time of life.

The pros and cons of this theory will be discussed later.

Lactation.

Success has been recorded in rendering defective lactation adequate with wheat germ oil (Shute, 1939), but there is little experimental evidence to support this. Cases of women with defective lactation, although receiving vitamin E, have also been recorded. Progesterone and testosterone, as well as vitamin E have been said to be of importance in the maintenance of breast milk.

It has been shown that human milk is rich in Vitamin E, if the mother's diet is well balanced. Sterility in Vitamin E deficient rats is cured if they are fed on this milk. (Muller, 1936.).

Although Shute reported a few successful cases, they were not of sufficient number for definite conclusions to be drawn. He also states that some cases, requiring wheat germ oil for a successful pregnancy, had an excessive secretion of milk just after delivery. One case produced eight ounces for each feed for several days, and some continued the excessive secretion for seven to ten days post-partum. These results might be due to the abnormally high quantities of Vitamin E being administered.

Of his patients on wheat germ oil, sixty per cent nursed their babies for three months or more, eight per cent showed defective lactation, and sixteen per cent an unusual amount of secretion. Of normal patients, fifty eight per cent nursed their babies, eighteen per cent showed signs of poor lactation, and seven per cent an unusual amount of secretion.

Oestrin is responsible for the proliferation of the duct system and the development of the nipple, but the mammotropic hormone of the anterior pituitary is responsible for the actual secretion of milk. It comes into full play when the oestrin of the body begins to be excreted in the puerperium, so if vitamin E is in a state of balance with the oestrogenic substance, one would expect excess of the vitamin to result in excessive secretion of milk. (Shute, 1938b.).

The Duration of Labour.

It has been found that the administration of wheat germ oil to pregnant women does not prolong labour. In some cases of abruptio placentae, Shute found that the administration of the oil up to the onset of labour in no way interfered with the induction of labour. He also could not stop labour in its first stage with massive doses of wheat germ oil, and saw no reason why vitamin E should interfere with labour by its antagonistic action to oestrogens, as no more than the usual ten per cent of normal women displayed the presence of his oestrogenic factor in the serum just after or during parturition. (Shute, 1938b.). Other authors confirm that there is no increase of oestrin found in the serum before labour. (Smith, Smith, 1937.).

Dysmenorrhoea.

Shute found that under treatment with vitamin E, certain cases of dysmenorrhoea, who showed an excess of his oestrogenic factor in their serum, were cured. He found the factor in forty nine per cent of cases of dysmenorrhoea. (Shute, 1940.). Others did not find vitamin E of value in the treatment of this condition. (Juhász-Shaffer, 1933.).

Vaginitis and Vulvitis.

Vulvovaginitis at the menopause has been ascribed to cessation of ovarian function, but Shute found that many cases were made worse with oestrogens. Many castrates, both post-menopausal and after X-ray treatment, showed in the urine oestrin, which may be derived from the pituitary or adrenal cortex, so these cases had probably a definite or excessive oestrogenic activity in their bodies. He found the presence of his oestrogenic factor in the serum of some of these cases, and under treatment with wheat germ oil, this factor disappeared, and the symptoms were cured. The action of vitamin E in genital hypoplasia may have some connection with the interference of pituitary function, which has been said to occur with vitamin E deficiency. (Shute, 1938a.).

Congenital Abnormalities.

It has already been suggested from experimental evidence, that an adequate supply of vitamin E might play a part in the prophylaxis of congenital abnormalities. (Macomber, 1933.); (Byerly, Titus, Ellis, 1935.). Under the conditions of vitamin E deficiency as defined by Shute, a slightly higher incidence than normal of the birth of deformed infants has been noted, and he quotes five cases, whose mothers showed the presence of the antiproteolytic factor in their serum during the first trimester. (Shute, 1939.).

It should also be realised that many abortions are associated with the delivery of abnormal foetuses, and

some think that because of this risk, the prevention of abortion with vitamin E is a misguided effort. However, there have been no significant reports of foetal deformities ~~with wheat germ oil~~ in infants from pregnancies supposedly brought to term with wheat germ oil. (Vogt-Möller, 1933b), (Watson, Tew, 1935b.). Shute recommends that if there is evidence of raised blood oestrogen, and therefore of vitamin E deficiency, as late as the end of the second trimester, it should be suspected at least that the foetus is deformed. (Shute, 1938.).

The results of various Authors' treatment of threatened abortion with wheat germ oil, and their incidence of congenital abnormalities may be tabulated:

AUTHOR.	THERAPY.	NUMBER OF MOTHERS TREATED FOR THREATENED ABORTION.	NUMBER OF MOTHERS DELIVERED OF VIABLE CHILDREN.	NUMBER OF CHILDREN WITH ABNORMALITIES DELIVERED.
Watson, Tew. 1935.	Wheat Germ oil.	21	14	0
Currie, 1938.	"	17	18	1
Cromer, 1938.	"	3	3	0
Shute, 1939.	"	23	19	3

It has also been considered that the neo-natal mortality rate is increased when there is a lack of vitamin E in the diet. (Browne, 1939.).

Miscellaneous Gynaecological Conditions.

It has been suggested that vitamin E might be of use in the treatment of amenorrhoea, menorrhagia, and of climacteric complaints such as hot flushes and headaches; ~~and~~ and in irregular glandular hypoplasia, which has been regarded as an ovarian insufficiency, due to lack of corpus luteum. There is no suggestive evidence that these conditions are due to deficiency of vitamin E, and the results of therapy have not been encouraging.

Nephritis.

The disappearance of signs of kidney damage, such as raised blood pressure and oedema, in certain cases of toxaemia of pregnancy under treatment with vitamin E, has raised the question of what part the vitamin plays in kidney function. No experiments have been carried out on this problem. However, Shute records a case of a woman with a history of acute nephritis, who during the sixth week of pregnancy developed a raised blood pressure and a slight albuminuria, with no obvious oedema. Under

treatment with wheat germ oil, the pressure fell to normal, and the albuminuria vanished. (Shute, 1939.).

(c.) Theories of the Mode of Production of the Effects of Vitamin E Deficiency in Man and in Animals.

In this section, the theories that have been brought forward to explain the action of vitamin E deficiency in the reproductive system will be discussed. The explanation of certain side actions of the deficiency, such as prolongation of pregnancy due to death of some of the foetuses, have already been discussed, so only theories which attempt to explain all its actions will be mentioned here.

First, it may be stated that the knowledge of the action of vitamin E in the physiological field is much less than that of its chemistry, and none of the theories are based on indisputable facts. The different way in which the lack of vitamin E affects the two sexes, and the absence of marked external manifestations of this lack, have offered many avenues of speculation as to the role of the vitamin. From the experimental evidence given, it is seen that lack of vitamin E results without doubt in such conditions as habitual abortion, and maldevelopment of the embryo, in the experimental rat, but it is not so certain that similar conditions in humans and other animals result from this deficiency. However, the theories must be discussed as they apply to both certain and hypothetical results of deficiency of the vitamin.

The Cytological Theories.

The possible rôle of vitamin E in the normal functioning of the cell nucleus, and in oxidation-reduction processes of the cytoplasm, have already been discussed.

If vitamin E was necessary for the normal health of all or certain cells, it would explain the inability of the female to carry pregnancy to term owing to the death of the developing embryo, and would be compatible with the difference found in the male and female under conditions of its deficiency.

Pituitary Dysfunction.

In order to account for the changes found in the female reproductive system under conditions of vitamin E deficiency, it has been suggested that the vitamin is necessary for the normal functioning of the anterior pituitary gland. It may be needed for the normal metabolism of the actual cells, or for the production of the hormones. The evidence for and against this theory will now be reviewed.

The cellular changes found in the pituitaries of vitamin E deficient experimental animals have already been described, and the fact that some authors found an increased, some an unchanged, and some a decreased gonadotropic activity of these glands, has been noted.

In support of the theory, it has been stated that lack of vitamin E produces somewhat similar changes in experimental animals as does hypophysectomy, and for that reason it has been called a nutritional hypophysectomy. It has also been maintained that the production of the gonadotropic hormones is promoted by vitamin E, and that lack of it reduces the production of these hormones and of prolactin. (Clark, 1940.), (Verzar, Kodas, 1931.).

The reported success in the treatment of undescended testicle and genital hypoplasia with both vitamin E and gonadotropic hormones, supports the relationship between the vitamin and the gland, as the latter is necessary for normal genital and testicular development. The vitamin may stimulate the production of the hormone. (Clark, 1940.).

Barrie supports this theory, and states that the failure of reproduction, lactation, and activity of the thyroid is due to the impaired supply of gonadotropic, galactogenic, and thyrotropic hormones. This seems to be substantiated by his histological findings in the anterior pituitary gland. In cases of vitamin E deficiency in animals, the basophil cells were vacuolated and degenerated. Castration in animals and man results in similar changes. (Ellison, Wolfe, 1934.), (McCallum, 1936.), (Geller, 1935.), Gierhake, 1935.). The acidophil cells were likewise affected, and these are associated in the rat with the activity of the thyroid, and in man with the control of growth. (Cushing, 1933.). It has also been noted that mice which are congenital dwarfs show no acidophil cells in their pituitaries. (Smith, MacDowell, 1930.). The site of the secretion of the galactotropic hormone is unknown. Also the appearance of Barrie's experimental rats deprived of vitamin E with their stunted growth and inactive thyroids suggests pituitary dwarfism, with secondary cretinism. The evidence of Barrie's experiments indicated that the galactogenic and thyrotropic hormones were affected before the gonadotropic hormones in states of vitamin E deficiency, because the rats were sometimes able to give birth to normal offspring, but were unable to rear them. The fact that the first stages of pregnancy are normal in vitamin E deficient animals, suggests that pituitary changes cannot be gross till a later stage. (Barrie, 1937a.). Other Authors confirmed these findings, and noted that anterior hypophysectomy of animals during pregnancy produces abortion and failure of lactation, although removal of the posterior lobe has no effect. (Firer, 1933.), (Houssay, 1935.), (Robson, 1936.). Finally, some state the morphological and functional alterations in the anterior pituitary of rats, and the secondary changes in their thyroids, can be alleviated by giving vitamin E. (Singer, 1935.).

On the assumption that vitamin E is necessary for pituitary function, and from the experimental evidence that it is needed for normal placental development, Mason

and others suggest it may play a rôle in the prolan-progesterone mechanism during pregnancy. (Mason, 1939.), (Young, 1937.), (Bishop, 1937.) . Certainly if this mechanism broke down, and the quantities of progesterone in the body altered, abortion would result. It may also account for the succesful results of treatment of such conditions as threatened abortion with prolan and progesterone, as well as by vitamin E.

Some authors found that ~~the~~ vitamin E given orally or by injection to experimental animals, resulted in premature oestrus. (Szarka, 1929.), (Verzar, 1929.), (Adder, Boltink, 1929.) . Early opening of the vaginal membrane, and uterine hypertophy, were also noted after these injections in non-castrated, but not in castrated, infantile rats and mice. (Verzar, 1931, 1932.).

In vitamin E deficient rats, Verzar noted a silky coat like that resulting from hypophysectomy, and maintains that the vitamin is either closely related in function to the gonadotropic hormones, or necessary for their synthesis in the body. (Verzar, 1931.) . He found that the abnormal conditions of the hair and a lowered basal metabolic rate, which was also sometimes present, were cured by vitamin E. (Verzar, Kodas, 1931.) . A similar disturbance in the hair of rats, and an alteration in the feathers of hens, have been noted by others, when these animals have been deprived of vitamin E. These changes have been ascribed to hypophyseal disturbance. (Gierhake, 1933, 1935.) . The exceptionally high incidence of spontaneous deciduomata, and the tendency for silk thread deciduomata to be larger than normal, in the sterile uterine horn of pseudo-pregnant rats deficient in vitamin E, is regarded as suggestive of hormonal disturbance, although it may be an atypical responsiveness of the uterus to this deficiency. (Evans, 1938.).

Sterility resulting from vitamin E deficiency has been claimed to be prevented or cured by the injection of the follicular hormone, (Bisceglie, 1929.), or by the injection of hypophyseal hormones from pregnancy urine, although lactation could not be restored by the latter, when it could be by liver injections. (Agnoli, 1930.), (Marchesi, 1935a.) . The fact that gestation is prolonged in vitamin E deficient rats in the same manner that it is prolonged if it occurs at the time of lactation, lends added evidence to the theory of pituitary deficiency in these animals, as the latter phenomenon is due to the hormonal influences from this gland. (Brambell, 1937.) . It is possible that these phenomena are both caused by impaired function of the corpus luteum deprived of pituitary stimulation, although there is no morphological evidence of this. (Barrie, 1938b.).

The evidence to be derived from the alterations in the male pituitary, from vitamin E deficient animals, is somewhat similar, to that described above, and no more conclusive. It will be recorded in detail later. It may also be noted here, that in states of vitamin B 1 deficiency, signs of toxaemia due to pituitary hyperfunction have been found. (Siddall, 1938.).

It has already been stated that evidence of thyroid deficiency has been found in vitamin E deficient rats. The suggestion was that this was secondary to pituitary dysfunction, so this problem will be further considered now. If these changes were due to lack of vitamin E, it would support this theory, and the experimental findings in animals leave no doubt that this is so. (Barrie, 1937a.). Also, patients suffering from diseases, possibly due to lack of the vitamin, often also complain of symptoms of hypothyroidism, such as menorrhagia, colitis, and poor cold tolerance. (Singer, 1936.).

However, it must be mentioned that the changes in the thyroid may be due to a primary lack of vitamin E, and have no connection with the pituitary. This would be established if vitamin E was shown to restore the normal function of the gland in vitamin E deficient hypophysectomised animals. (Singer, 1936.) The effect of increased or decreased metabolism on the oestrin content of the body, and therefore indirectly on the gonad stimulating power of the pituitary, has also been described. Increased metabolism eliminates oestrin, and therefore increases the power. So certain pituitary changes might be secondary to thyroid ones.

The evidence against vitamin E interfering with the function of the pituitary will now be considered. Many authors have failed to demonstrate cell changes or alterations in the weight or volume of the anterior pituitaries of pregnant or non-pregnant vitamin E deficient rats. (Nelson, 1933.), (Muller, Muller, 1935.), (Euler, Zondek, Klussmann, 1933.). Others found that the sterility of these animals was unaffected by the follicular hormone (Csik, 1932.), or by corpus luteum or anterior pituitary extracts and implants, or by prolan A. (Nelson, 1931.), (Diakov, Krizenecky, 1935.), (Geller, 1934.), (Drummond, 1939.). It is suggested that these hormones are essential for the first phase of embryonic development, and account for the normal oestrus, ovulation, and implantation in vitamin E deficient animals, but that the vitamin is needed for further embryonic development and the completion of gestation.

Diakov found no evidence of gonadotropic activity in the form of precocious sexual development, or alterations in external genitals, ovary or uterus, when vitamin E was

given to infantile female rats intra-peritoneally. Anterior pituitary hormone given in a similar manner did result in sexual development. (Diakov, Krezenecky, 1933a.). Drummond also found that it had no effect on immature female rats, or on the ovaries, uterus, or vagina of hypophysectomised adult ones. (Drummond, Noble, Wright, 1939.) . Evans reports that in rats, on giving vitamin E, there is no significant increase in the proportion of young weaned, or in their weights, which would indicate that it had lactation-promoting properties. (Evans, Burr, 1928.).

The conflicting reports on the hormonal content of the pituitary in states of vitamin E deficiency, render the results of these experiments open to severe criticism. It may be that this content varies considerably under normal conditions, and a decreased content may not mean a decreased output. The discovery that the hormonal content of the glands varied between the two sexes when they were deprived of vitamin E, and that hypophyseal injury affects the ovary and testis alike, which vitamin E deficiency does not, is suggestive that changes found in this gland may be secondary to the disturbed function of other endocrine glands, rather than being the primary cause of pathological states of the reproductive system. (Evans, 1932.) . The fact that embryos of vitamin E deficient female rats begin to show pathological changes at the time that the anterior pituitary relinquishes the maintenance and the function of the corpus luteum in favour of the placenta, is also against the pituitary being primarily affected. (Astwood, Greep, 1938.).

This conflicting experimental evidence seems to indicate that, if there is an impaired gonadal-hypophyseal relationship in states of vitamin E deficiency, it is more justifiably regarded as a resultant than a cause of reproductive abnormalities characteristic of this condition.

This question can only be cleared up when further experiments have been carried out.

Disturbance of the Ovarian Sex Hormones.

Other Experimentors have suggested that the physiological picture in vitamin E deficiency may be one of hormonal imbalance, or disturbed production of the ovarian hormones, rather than a pituitary deficiency. However, the endocrine glands are so closely connected, that it is almost impossible to say whether the pituitary changes are primary to changes in the other sex glands, or vice versa. It seems impossible that a hormonal imbalance could occur without involvement of the pituitary, but it is possible that vitamin E is needed primarily for the production of ovarian hormones.

The favourable evidence for this theory will be given first. Some Authors state that vitamin E controls the production of oestrin and have found that excess of it produces oestrus in infantile or castrated female rats. (Beaumont, Dodds, 1941.)(Verzar, 1929.).

Others found that the effects of vitamin E deficiency in animals were prevented or cured by ovarian grafts, or injections of placental extracts. (Marchesi, 1935b, 1935c.) . Although this is an exception, some Experimentors demonstrated irreversable degeneration of the ovary in vitamin E deficient animals. (Underhill, 1939.).

Vøgt-Möller noted that artificial abortion in cows could be produced by digital compression of the corpus luteum per rectum and also by removal of it and of the ovary. If the ovary of mares is removed in the first half of gestation, abortion results, but usually not if it is removed in the second half. The corpus luteum of cows persists post-partum, but that of mares atrophies at mid-term. These findings indicate that abortion does occur if the animals are deprived of the ovarian hormones, oestrin and progestin. It is suggested that this takes place when vitamin E is deficient. (Vøgt-Möller, 1939.). Now that the amounts of these hormones excreted in the urine can be tested for, further light should be thrown on the effect of the supply of vitamin E on their metabolism.

The vitamin seems most closely allied in chemistry, clinical features and significance, and in physiological properties, to progestin; and habitual abortion can apparently be equally well treated with both these substances. They are both found in association with lipoid substances in plants and animals. For this reason it is possible that their actions are identical; but it is more likely that the vitamin is essential in the early months of pregnancy for the formation of the placenta, which produces the luteal hormone in the later months. Young suggests that the work of the vitamin is completed when the placenta is fully developed. (Young, 1937.). Certainly after this time, abortion does not always result after removal of the ovary, which it does after its removal at an earlier date, and the corpus luteum diminishes in size. The absence of progestin results in a toxic condition with albuminuria and accidental haemorrhage, not unlike that reported by Shute to occur in experimental vitamin E deficient animals. Watson goes as far as to suggest that certain glandular extracts may owe their effectiveness to the accidental presence of vitamin E in the preparation used. (Watson, 1936.).

The possibility that vitamin E might play a vital part in the synthesis of the vitamin has also been raised.

The experimental evidence seems to indicate that vitamin E controls the production of the ovarian hormones through the pituitary, and not by a primary interference with their metabolism. (Collins, Weed, Collins, 1940.). Drummond failed to restore fertility in vitamin E deficient rats with implants of progesterone or testosterone, which was in sharp contrast to the effect of the former in cases of threatened abortion in women. (Drummond, 1939.). The fact that oestrus and ovulation are normal in these animals, is also against ovarian hormones being grossly affected. Other Authors failed to cure vitamin E deficiency sterility with either oestrin or progesterone, although they may have been used incorrectly. (Geller, 1933.). (Nelson, 1931.). (Csik, 1932.).

In vitamin E deficient animals the lack of pathological changes in the maternal placenta, and the time of onset of changes in the embryo after the placenta has been formed, are against a relationship between vitamin E and progesterone, unless the latter exerts a specific influence on the developing ovum. (Vøgt-Möller, 1939.). Hypophysectomy in the second half of pregnancy does not cause premature atrophy of the corpus luteum. (Selby, Collip, Thomson, 1933.). This may explain the normality of this gland, although in the same animal the pituitary shows gross changes. This normality in turn suggests that placental deficiency may not be a contributory factor to resorption. (Rowlands, Singer, 1936.). However, lack of vitamin E does interfere, apparently, with placental function to some extent.

There is really no close chemical resemblance between vitamin E and the sex hormones, and it is unlikely that it plays a part in their synthesis. It has been found that in experiments which showed that vitaminE had a stimulating action on the sexual cycle, this only took place if the ovaries were present and functioning normally. Anterior pituitary extracts likewise needed the presence of the ovaries for their action, but oestrin itself did not, (Verzar, 1929.). The fact that vitamin E cures conditions due to its lack in animals, while showing no oestrogenic or luteinising properties of its own, also supports the view that they are not closely related. (Saphir, 1936.).

Bacharach found that vitamin E had no effect on the ovarian cycle of mice, although he does not know if this applies to other animals such as rats, in which positive results have been recorded. He injected 10 milligrams of dl.-tocopherol acetate into each animal. (Bacharach, 1940.).

Most Experimentors have found no demonstrable change in the ovaries of experimental vitamin E deficient animals.

From the results of these experiments, it seems to be evident that vitamin E does no more than supplement the

the work of the hormones, and that it is not essential for their normal functioning, anyhow early in pregnancy.

The Relationship of Vitamin E to the Oestrogen Content of Serum.

Of all the theories to explain the effects of vitamin E deficiency, that which expounds an hormonal imbalance with a dominant action of an oestrin-like substance, is the most comprehensive. It was suggested by Shute. The experimental data in animals and man on which he bases his facts has already been given. It may be remembered that in states of vitamin E deficiency, he found in the serum an antiproteolytic factor, which he identified as oestrin or an allied substance.

Experimentors found that in pregnant animals, follicle ripening, and even ovulation were produced during pregnancy, by injections of anterior pituitary hormones or implants, and somewhat similar changes were produced by gonadotropic preparations from the urine. (Seyle, Collip, Thomson. 1933), (Katzman, Levin, Doisy, 1931.).

Changes in the pituitary were also produced in non-pregnant animals by oestrogenic ~~hormones~~ and gonadotropic hormone (Nelson, 1934.). Shute suggests that all these effects are due to oestrin produced by the animals' own gonads under stimulation of the various other hormones, and this would account for some of the changes seen in the ovaries and pituitary in states of vitamin E deficiency. The production of thyroid hypoplasia by the injection of oestrogens into animals has been noted (Benazzi, 1933.), and it has also been cured by wheat germ oil. (Paal, Kleine, 1933.). A similar hypoplasia is seen in states of vitamin E deficiency. It has also been seen that on injecting hypophyseal extracts into pregnant animals, death of the embryo, resorption, bloody amniotic fluid, and placental detachment occur. This later phenomenon is allied to abruptio placentae in pregnant women. (Teel, 1926.). A similar picture is found on injecting oestrin, (D'Amour, D'Amour, Gustavson, 1933.) and when there is lack of vitamin E, ~~in the above experiments~~ as in the above experiments, so probably the hypophyseal extracts also act by liberating oestrin. In support of this, Shute injected gonadotropic hormones into pregnant females in whom therapeutic abortion was desired on account of other diseases, and found a transient appearance of his oestrogenic factor in the serum. That oestrin can produce death of the embryo and sterility, is thus confirmed, and it has been previously suggested that in pregnancy there might be a balance between it and anterior pituitary gonadotropic hormone. (Leonard, Meyer, Hisaw, 1931.). A high excretion of oestrin in the urine in cases of abortion has been noted, (Murphy, 1933), and oestrin has a definite action on the onset of labour. (Robson, 1934.). Now Shute maintains that during pregnancy, oestrin and vitamin E are also in a state of balance, and if there is lack of the latter, there is an unopposed action of the former. It can be seen from the experiments quoted

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above, that this can give rise to/variety of results, as the pituitary hormones, progestin and oestrin are in a delicate balance. The disturbance of this balance produced by injecting these various hormones may account for some of the conflicting reports recorded in the last two sections. The main action of this excess of oestrin is to produce abortion, and Shute maintains that this is due to an antiproteolytic property of the oestrin which resists the digestive action of trypsin. The trophoblast excretes trypsin in order to dissolve the maternal tissues, so that the ovum can be securely implanted. If there is excess of the antiproteolytic factor in the mothers' serum this is impossible, the ovum is insecurely attached and abortion is liable to occur, or at a later stage, abruptio placentae. The poor attachment of the villi as a cause of the latter condition has been confirmed by other authors. (McGlinn, Harer, 1935.).

As has been mentioned before, Shute has found this factor in the serum of women suffering from many obstetrical and gynaecological diseases, possibly due to the influence of excessive amounts of oestrin. Under treatment with vitamin E, the clinical condition improved, and at the same time the oestrogenic factor disappeared from the serum. It returned when the vitamin E was stopped. His best results were in cases of threatened abortion and abruptio placentae, and the factor was present in the serum of seventy per cent of all his cases suffering from con of the former condition. He also reports successes in cases such as dysmenorrhoea, climacteric complaints and senile vaginitis. He does admit that this is not the only cause of abortion, which may account for the twenty to thirty per cent of failures of other workers. He also states that you may get premature delivery while on wheat germ oil, and that in some cases there is not a perfect correlation between the temporary removal of the factor from the serum and the cessation of the signs and symptoms of the premature termination of pregnancy. It is possible that other dietary factors play a part. (Kudyashov, 1933.)(Olcott, Mattill, 1934.). (Rosahn, Green, 1934.).

resistance to trypsin

The theory has been criticised on various grounds. Cuthbertson and Drummond could not find any anti-trypsic activity in the serum of vitamin E deficient rats, when testing it with a solution of gelatin and trypsin, and suggest that Shute's responses are probably artefacts. They state that his tests are invalid, and that the production of acid, which is taken as evidence that digestion due to trypsin has occured, is due to some other cause. Their reasons for this are that there is no initial increase in the formaldehyde titration, or inactivation of the trypsogenic activity by heat. (Cuthbertson, Drummond, 1938.).

Shute argues that it was known years ago that the absence of an initial increase in the formaldehyde titration was a characteristic of tryptic digestion. (Fine, 1931.) . Moreover, this phenomenon is only concerned with the liberation of amino acids in the later stages of digestion, while in his test it is not pertinent to its technique, as no amino acids may appear within the period of observation. As to the inactivation of trypsin by heat, although the digestion occurred over a temperature range of 20° C to 90°C, it only took place for forty minutes, and the preparations were crude and acid in reaction. It takes a temperature of 65°C forty minutes to inactivate pure preparations. (Eddie, 1914.). He mentions that the eventual inactivation of the antitrypsin by the heat may be an explanation of the phenomena observed, as digestion, although delayed does finally take place. (Shute, 1940a.).

Cuthbertson and Drummond also criticise the technique of his tests, and suggest that his findings may be due to artefacts such as absorption of carbon dioxide. Shute replies that it may be difficult to get an end-point to the titration using phenol phthalein as an indicator, owing to the gradual rise of the acid, but it is a better one than that used by these Experimentors. He finds it difficult to see how absorption of carbon dioxide could account for such variation in the acid content of the digest as he observed, owing to the fact that the tubes were small, half full, and tightly corked. He finds it more difficult to believe that trypsin has been in contact with serum protein for over two hours, and produced no digestion, than that it has done so. He mearly states that this digestion is delayed.

Drummond, Noble, and Wright, criticise Shute's theory on the grounds of inadequate experimental evidence. They state that prolonged exposure of the female organs to an

excess of oestrin would not allow of the normal occurrence of oestrus, ovulation, and implantation, as well as the first stages of pregnancy. They found that a small quantity of oestrin given to female rats prevented implantation, although large doses did not interfere with pregnancy once it was established. They noted no disturbance of the oestrus cycle, or decrease in the weight of the ovary, or increase in the weight of the adrenal glands or pituitary in vitamin E deficient animals, as would be expected if there was an excess of an oestrogenic factor in their serum. The testicular changes observed in vitamin E deficient male animals are also different from those seen after prolonged administration of oestrin. Similar effects to those of vitamin E deficiency were not observed in rats given large doses of oestrogens. (Drummond. Noble. Wright. 1939.).

Shute in reply argues that there is adequate physiological evidence to support the theory. Vitamin E is not Oestrogenic, and other Experimentors have found that giving oestrogens to female rats for long periods does not interfere with the oestrous cycle or impair fertility. The ovarian and uterine weight alters under this treatment but it soon returns to normal. (Wade. Dorsy.1935.). The progesterone-like actions of vitamin E can be explained by the fact that both substances are antagonistic to oestrin. This antagonism may be an additional cause for the occurrence of abortion, acting in concurrence with the resistance of the serum to trypsin, as it is known that a reduction in the amount of progesterone results in abortion. Thecorpus luteum is still active in rats on the tenth day of pregnancy, which is compatible with the curative effects of vitamin E on that day. (Bacharach.1939.) . He also states that the high oestrogen content of the serum is not incompatible with the changes in the pituitary or testicle.

He is not able to explain why similar changes to vitamin E deficiency are not produced by giving oestrogens to animals, or why these are not counteracted by anti-oestrogens such as pituitary extracts and progesterone. He suggests that the question must be considered in relation to the varying and specific character of the different oestrogens. It is also possible that in states of vitamin E deficiency there has been a mobilisation of the oestrogens for a long period so that they are acting with relatively no opposition; while

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in normal animals, when oestrogens are suddenly given, a compensatory mechanism with the production of progesterone and corticosterone comes into play, so one would not expect the same changes to occur. It must also be borne in mind that the various male and female sex hormones show various degrees of oestrogenic and anti-oestrogenic activity , and that the natural oestrogens of the body may differ in total effect from the effect of the forms used in injection experiments.

The fact that some Experimentors have produced oestrus in immature female rats with wheat germ oil need not invalidate this theory, as the reports on these results have been very conflicting. (Shute.1939, 1940a.).

In support of the presence of his factor in the serum of women, supposedly suffering from vitamin E deficiency, which he found as the result of laboratory experiments, Shute records a case of a woman who at the sixth week of pregnancy showed a negative Friedman's Test, presumably due to a high blood oestrogen content. Eight drams of wheat germ oil were given as a single dose, and twenty four hours later the Friedman's Test was TypeII. Then a second dose of six drams of the oil was given, and twenty fours later the Friedman's Test showed a maximum positivity. The suggestion is that the oil controlled the blood-oestrogens, and so permitted prolan to escape and act on the rabbit's ovaries used in the Friedman's Test. (Shute.1939.). He has also found that on giving wheat germ oil to a patient, her uterus did not involute normally, and he believed this was due to the neutralising effect of the oil on oestrin which is responsible for the contraction of the uterus. When the oil was stopped, and oestrin and pituitrin given, the uterus involuted normally. (Shute.1937c.).

If this theory is established as correct by further experimental and clinical evidence, the test for an anti-proteolytic factor in the serum will be of great value in the diagnosis of conditions which may be due to vitamin E deficiency. The test will also be of value in controlling treatment, as this cannot be adequate if the factor does not disappear from the serum. It may be noted that some Authors quote successes in the treatment of certain diseases with doses of vitamin E far smaller than Shute found necessary for the removal of his factor. Others have already reported the successful use of the test for diagnostic and clinical purposes. (Cooper.1939.).

However, at the moment, the hypothesis stands or falls on the validity of the test for an antiproteolytic factor in serum of vitamin E deficient animals. It also depends on several assumptions. It takes it forgranted that the proteolytic ferment of the embryonic villi is trypsin, and that the strength of this substance at the villous surfaces is comparable to that in the experimental solutions. The factor has never been isolated chemically from the blood or the urine, although it is perhaps excreted in a modified form in the latter. Until some of these questions are cleared up, it can only be stated that, as far as the reproductive system in vitamin E deficiency is concerned, it does not appear to be completely incompatible with any of the changes observed.

(d). The Application of Vitamin E to Clinical Medicine.

1. Habitual Abortion.

A seventy to eighty percent cure-rate for cases of habitual abortion treated with vitamin E is claimed by various authors.

Vøgt-Möller was the first to try the vitamin in this field. His first report was of two women who had had four and five miscarriages respectively following one normal pregnancy. He administered 5c.c. of wheat germ oil daily for two weeks, followed by 5c.c. on alternate days for two weeks, and then 5c.c. every sixth day. Both were delivered of living children. He noted no abnormalities in the mothers' diet. (Vøgt-Möller, 1931.).

His next report was of two series of cases. In the first series he gave forty drops of wheat germ oil, in the form of Fertilan, three times a day to twenty cases of habitual abortion. The treatment was continued for four months, from the third to the seventh month of pregnancy, and was followed by the administration of a dessertspoonful of wheat germ oil three times a day. Seventeen of the cases were delivered of living children. In the second series the treatment was the same. The cases showed no anatomical or physiological abnormalities. The husbands had normal spermatozoa, and the wives revealed nothing abnormal on gynaecological examination, or on serological or hormonal tests. As a result of the treatment, thirty-eight living children resulted from fifty two pregnancies in women who had had previous abortions. (Vøgt-Möller. 1933a, 1933b.).

Finally, he recorded fifty six cases of habitual abortion treated with wheat germ oil, with a successful outcome of their pregnancies in thirty eight of them. (Vøgt-Möller. 1936.).

Currie reports on three series of cases. In the first, there were twenty nine cases of habitual abortion. Under treatment with one to three minims of wheat germ oil daily by mouth, with vitamins A and D in addition, for an average time of five months, twenty three of them completed a normal pregnancy. This group had had seventy three previous pregnancies resulting in only eleven living children, five of whom died in infancy. The six remaining cases of this series had uneventfully passed the sixth month of pregnancy. (Currie.1936.).

In the second series, there were thirty seven women who had had more than one previous abortion. They were treated with vitamin E in the form of one capsule, containing three minims of wheat germ oil extract, daily. In addition, vitamins A and D and calcium, were given to some of the cases, and the treatment was continued from the third to the sixth month to the end of pregnancy. A careful examination was carried out of each of the women, and no signs of fibroids, retroversion, torn cervix, syphilis or general diseases were found. Two of them did have a positive Wassermann Reaction. Previous to treatment they had had a hundred and thirty pregnancies, which resulted in only sixteen viable children, but after treatment, thirty five gave birth to children, and there were two cases of ~~child~~ twins. The other two women aborted at fifteen and twenty weeks respectively after the start of pregnancy. Four of the children died in hospital from prematurity. Twenty five of the mothers were delivered at full term, one at thirty nine weeks, three at thirty eight weeks, two at thirty six weeks, one at thirty four weeks, one at thirty three weeks and two at thirty two weeks, after the start of pregnancy. Five of them showed gross signs of toxaemia, which made it necessary in one case to induce labour by puncturing the membranes, and in two others to perform Caesarian section. Prolutan was tried in the treatment of this condition. Two of the other cases had to be delivered by forceps and one by Caesarian section. In all except six of the cases,

treatment was not started till after the twelth week of pregnancy, when the placenta was fully formed, but the success of the treatment seemed to be sufficient for credit to be ascribed to the use of the vitamin, even after this has occured. It is recommended that the dose of vitamin E never be smaller than three minims of the oil extract daily. (Currie.1937.).

In the third series, which was divided into three sets of cases, the patients received one three minim capsule of wheat germ oil extract daily, containing six milligrams of tocopherol . Sometimes the treatment was not started till the middle of pregnancy, but was still successful. Progesterone was found to be a valuable aid in treatment, as were preparations of the luteinising hormone. If uterine infection was found in any of the cases, curettage was carried out and conception attempted immediately afterwards. The first set of cases consisted of eighty one women, who had had two hundred and seventy four previous pregnancies, with only forty seven viable children. They received treatment for from three to thirty two weeks, with an average of eighteen and a half weeks, and as a result there were sixty four live births with sixty two viable children. There were two monsters. There were six premature births, twelve stillbirth, and five abortions. The second set were eight women, who had had twenty nine previous pregnancies, only five of which were successful. They were treated for from ten to thirty weeks and all gave birth to viable children, although one was premature. The third set also consisted of eight cases, and under treatment they gave birth to seven children, one of whom was premature. In the other case the embryo died in utero. (Currie.1939.).

Watson treated his cases of habitual abortion with wheat germ oil, after it had been tested for potency on rats. The optimum dose was not known, so 3 to 6c.c. of the oil were given daily by mouth. Treatment began as soon as pregnancy was recognised, and even before this in two cases, and was continued well beyond the usual time of abortion, sometimes up to the start of parturition. No gynaecological condition was found in any of the patients, and abortion was defined as a spontaneous cessation of pregnancy at any stage, which resulted in the delivery of a dead foetus. In his completed list there were forty six cases and these were divided into two groups. The first group had had two or more previous abortions. Of these, eighteen had each had three to fifteen previous abortions, and under treatment thirteen of them went to term or

nearly so; ten for the first time. There was one case of accidental haemorrhage and abortion, and one of spontaneous abortion occuring twenty five days after the start of treatment. The rest of the group numbered seventeen, and had each had two previous abortions. After treatment twelve went to term, but there were five spontaneous abortions, with a definite toxic state in one case. The second group consisted of women who had had only one previous abortion. They were eleven in number, and after treatment there were nine viable children born. One aborted soon after treatment was started, and there was one miscarriage, and some had threatened abortions but recovered and proceeded to term.

Watson states that these results confirm the value of vitamin E in the treatment of habitual abortion, but admits that his second group of cases is not of much significance. He thinks some of his failures may have been due to the fact that one of the three sources of the wheat germ oil used in these experiments was inferior in potency to the others. (Watson, 1936), (Watson, Tew, 1936.).

Tew treated twenty seven cases of habitual abortion with vitamin E. Each case had had one to ten previous abortions, but under the possible influence of the vitamin there were nineteen successful pregnancies, three abortions and five expectant cases. (Tew, 1934.).

MacDonald examined cases of habitual abortion for such conditions as uterine displacements, fibroids, and deep cervical lacerations and, when these were not present, treated them with wheat germ oil with progesterone and arsenicals in some cases. The treatment was started as soon as possible after the start of pregnancy, but this was usually after placentation was completed. The oil was given in a concentrated form in three minim capsules, under the trade name of viteolin; one capsule being given three times a day. He divided the women into two groups, those who had had two or more previous abortions and those who had had only one. There were eighteen cases in the first group, and they received treatment for three to thirty four weeks, with an average of seven and a half weeks. Two of the cases also received arsenicals as well, as there was a suspicious history of syphilis, although the Wassermann Reactions were negative. Two of the cases developed signs of late pregnancy toxaemia while

under treatment, but this may have been of the eclamptic type. The patients had had fifty three previous pregnancies, with only nine live births, of which three were premature; and four of the babies died in infancy. After treatment, seventeen of them gave birth to living children, and one aborted after two weeks of treatment. There were no stillbirths or monsters, but two of the infants were premature. The second groop consisted of four cases, and they received treatment for eight to twenty four weeks, with an average of seventeen and a half weeks. They had had six previous pregnancies amoung them, none of which resulted in living children. However, after receiving vitamin E, two of them gave birth to viable children, one aborted, and there was one stillbirth, possibly due to a toxic state occurring in the mother. There were no monsters.

MacDonald suggests too large doses of vitamin E should not be given, in case the protective effects of oestrin are overinhibited, and a hydatidiform mole results. (MacDonald, 1939.).

Describing his nutritional work, McGonigle mentions the use of wheat germ extract in preventing miscarriage. He selected seven women whose last pregnancy had terminated in a miscarriage and gave them one capsule of the extract daily, over periods varying from three to six months. In each case, a full-time live child was born. He also mentions treating forty eight children, suffering from the presence of threadworms in the bowel, with a similar dose of the extract for the period of one week. The treatment was successful in thirty four of these cases. (McGonigle, 1935.).

In a series of two thousand six hundred and eighty seven pregnancies, Bishop found the incidence of spontaneous abortion was 3.7 percent, and the incidence of habitual abortion only 0.41 percent. He reported twenty two successful pregnancies in eighteen cases of habitual abortion treated either with progesterone or vitamin E. Three of the women had a second pregnancy which went to term without treatment, so he suggests that the single administration of these two substances has a lasting effect, possibly permanently correcting defects in the endocrine mechanism. (Bishop, 1937.).

Weed and Collins treated twelve cases of habitual abortion with cold pressed wheat germ oil. They were given one to one and a half drams of the oil daily till

the middle of the eighth month of pregnancy, and in addition received intramuscularly one cubic centimetre of the anterior pituitary-like hormone, Antuitrin S, every week till the middle of the fourth month of pregnancy. Some of the patients received one rabbit unit of progesterone intramuscularly once a day, if they suffered from cramp or if there were signs of bleeding, and one tenth to one half of a grain of dried thyroid twice a day, if they showed signs of hypothyroidism, such as obesity, irregular periods, or slow pulse. Under this treatment, eight of the women were delivered of normal full-term healthy children, and four were six and a half months pregnant at the end of the experiment. It is difficult to assess these results, owing to the multiplicity of therapeutic agents used. (Collins, Weed, Collins, 1940.).

Juhász-Shaffer reported five cases of habitual abortion, who were all delivered of living children after treatment with wheat germ oil. (Juhász-Shaffer, 1933.).

Barker, as a result of clinical observations, recommends giving 3 to 6 c.c. of wheat germ oil orally, as soon as pregnancy is recognised, and continuing this dose until the onset of labour. It may be increased up to 20c.c. if threatened abortion supervenes. (Barker, 1939.).

Succesful results have also been recorded by other Authors. (Gierhake, 1933.), (Tanberg, 1936.), (Martius, 1937.).

Shute considers the vitamin is of more value in the treatment of threatened abortion than of habitual abortion, but he has found it of some value in the latter condition. However, he records the occurrence of seventeen abortions, two miscarriages, and four premature births among one hundred and eighteen women who had completed pregnancy while under treatment with wheat germ oil. (Shute, 1939.).

Finally, Browne suggests that these results recorded above are of little value, as no control observations were made of similar patients, who were not receiving treatment. In support of this, he mentions eighteen of his patients, who were suffering from habitual abortion, having had three previous unsuccesful pregnancies in most cases, eight of whom received progesterone, three vitamin E, and seven nothing but good advice. All the women in the last group gave birth to living children. (Browne, 1938b, 1939c.).

All these reports may now be tabulated:

NAME OF AUTHORS.		NUMBER OF CASES TREATED.	NUMBER OF LIVING CHILDREN DELIVERED.
Vøgt-Möller,	1.	2.	2.
	2.	20.	17.
	3.	52.	38.
	4.	56.	38.
Currie,	1.	29.	23.
	2.	37.	35.
	3a.	81.	64.
	3b.	8.	8.
	3c.	8.	7.
Watson,	1.	35.	25.
	2.	11.	9.
Tew.		27.	19.
MacDonald,	1.	18.	17.
	2.	4.	2.
McGonigle.		7.	7.
Weed and Collins.		12.	8.
Juhász-Shaffer.		5.	5.
Total.		502.	324.

This gives a combined cure rate of only sixty five per cent, which is little more than one would have expected in these cases if no treatment had been carried out. However, in several of the reports of the various Authors, some of the women had safely passed the usual time when abortion occurred, although they had not completed their pregnancies.

2. Threatened Abortion.

In most cases, the investigations on the value of Vitamin E in the treatment of threatened abortion were carried out by the same Investigators, and on patients under the same conditions as those which were carried out for habitual abortion. For that reason, if references to similar Authors are given, certain details, such as the examinations carried out on the patients prior to treatment, will not be repeated.

Currie treated sixteen cases of threatened abortion, over periods varying from thirteen days to twenty four weeks, with one capsule, containing three minims of wheat germ oil extract, daily. They also received other recognised methods of treatment. Forteen of them went to term and were delivered of viable children; eleven

went to full term, two to the thirty eighth week of pregnancy, and one to the thirty sixth week. One of the failures was suffering from a vesicular mole. He recommends that in the present state of our knowledge it is safer to give vitamin E throughout pregnancy, to guard against the possibility of threatened abortion occurring. (Currie, 1937.).

He also gave similar doses of wheat germ oil, and, in some cases, progesterone or luteinising hormone, also, to forty other cases. The treatment was continued from the time the threatened abortion was first recognised, for from four to twenty two weeks. Thirty six of the women recovered and were delivered of viable children, only one of whom was premature. There were three still-births, and one of the embryos died in utero. (Currie, 1939.).

Watson reported on the treatment of nineteen cases of ~~treatment~~ threatened abortion with wheat germ oil, and other recognised therapeutic agents. The treatment was not started till after the symptoms of this condition had been recognised, and in the majority of cases bleeding had already begun. The oil was given by mouth, usually in doses of 3 to 6c.c. daily, but sometimes as much as 20 c.c. was given in one day. As a result thirteen of the cases went to term. The remainder aborted. Watson considers that Vitamin E may well be of value in this condition, but does not think his results are of much ~~value~~ use, as the patients were getting other treatment as well as the vitamin, and some of the cases were too advanced for any kind of treatment to benefit them. One of his sources of wheat germ oil was also of very low potency. (Watson, 1936.), (Watson, Tew, 1936.).

Tew treated three cases of threatened abortion with wheat germ oil. Two completed their pregnancies normally and one aborted. (Tew. 1934.).

MacDonald gave massive doses of wheat germ oil to twenty cases of threatened abortion and antepartum haemorrhage. The treatment was continued for three to thirty two weeks, with an average of thirteen weeks, from the time when bleeding commenced. In a few cases the bleeding had continued for as much as ten days before treatment was started, although usually it had not done so for more than two and half days. The patients among them had had thirty previous pregnancies, which had resulted in twenty one viable infants. In their present pregnancies, of the women nine recovered and were delivered of viable children, although two were premature and one died in infancy; five are in good health and still continuing their pregnancies; and five aborted. There was one case of a vesicular mole,

whose uterus was evacuated owing to persistent loss of blood. There were no stillbirths or monsters. (MacDonald, 1939.).

In a series of a hundred and fifty pregnant patients, Shute records the successful treatment with wheat germ oil of twenty three cases of threatened abortion, and seventeen of threatened miscarriage. Among these there were three patients who were habitual aborters; and two others were delivered of twins at term. He had eighteen failures, but there was one case complicated by malignancy, and one was a habitual aborter. There were no cases of retained placenta. He does not diagnose any case as one of threatened abortion, until two of the three signs of uterine haemorrhage, uterine contractions, and low sacral backache are present, when none were present before. (Shute, 1939.).

Weed and Collins gave eight to twelve drams of wheat germ oil in twenty four hours to their patients at the first signs of a threatened abortion, and then continued with one to one and a half drams daily, till about the eighth month of pregnancy. They report on twenty four cases who received this treatment, fourteen of whom were delivered of normal full-term children, and the remainder who recovered from their symptoms and are continuing their pregnancies normally. Some of them also received anterior pituitary hormone, thyroid or progesterone. The doses of these substances have already been given in a reference to these Authors' work in the previous section.(Collins, Weed, Collins, 1940.).

Cromer succesfully treated three cases of threatened abortion with wheat germ oil. (Cromer.1938.).

These results can now be summarised.

NAME OF AUTHOR.	NUMBER OF CASES TREATED.	NUMBER OF CASES RELIEVED OF SYMPTOMS.
Currie, 1.	16.	14.
2.	40.	36.
Watson.	19.	13.
Tew.	3.	2.
MacDonald.	20.	14.
Shute.	58.	40.
Weed and Collins.	24.	24.
Cromer.	3.	3.
Total.	183.	146.

This gives a combined cure-rate of about eighty one percent, which at first sight seems to indicate that Vitamin E is of definite value in the treatment of this condition. Unfortunately, most cases received other forms of treatment at the same time as the vitamin, so it cannot be judged which was the successful therapeutic agent.

3. Sterility.

Vøgt-Möller stated that he cured two out of five cases of sterility with wheat germ oil. He gave forty drops of the oil three times a day, ~~from the third~~ till the seventh month of pregnancy, and followed this with a dessertspoonful of wheat germ ~~oil~~ three times a day for the rest of pregnancy. (Vøgt-Möller, 1933a.).

Watson records fifteen cases of sterility treated with wheat germ oil to facilitate impregnation. Six were cases of primary sterility, and eight had had one or more abortions. Only two had previously had a living child. However, no pregnancies occurred after treatment, and he does not think the vitamin is of any value in the treatment of human sterility. (Watson, 1936.), (Watson, Tew, 1936.).

Tew reported no successes on the treatment of ten cases of sterility with wheat germ oil. Six of these were cases of primary, and four of secondary sterility. (Tew, 1934.).

These results may be tabulated as follows:

NAME OF AUTHOR.	NUMBER OF CASES TREATED.	NUMBER OF CASES BECOMING PREGNANT AFTER TREATMENT.
Vøgt-Möller.	5.	2.
Watson.	15.	0.
Tew.	10.	0.
Total.	30.	2.

This indicates that vitamin E cannot be of much value in the treatment of this condition.

4. Abruptio Placentae, & Toxaemia of Pregnancy.

Animal experiments indicate that the vitamin might be of value in the treatment of these conditions.

During his studies on the treatment of cases of habitual abortion with vitamin E, Shute found that sixty five per cent of these cases showed signs of abruptio placentae before treatment was started, and seventy five per cent of them had an excess of his antiproteolytic factor in their serum, which indicates they may have been deficient in the vitamin.

Vitamin E in adequate doses, such as eight to twelve drams of wheat germ oil in twenty four hours, abolished the signs and symptoms of this condition, such as uterine tenderness, uterine cramp, and sacral backache, in twenty hours. They returned when the vitamin was stopped. It also seems to stop further placental separation once this has started, as uterine haemorrhage stops under treatment almost as soon as the symptoms. (Shute, 1937a, 1937b.).

He also reports success in the treatment of toxaemias

of pregnancy with vitamin E, if they are treated early enough, and found that signs such as an increase in weight, oedema, and albuminuria, disappeared. If the treatment was started later, it held the condition in check, but did not cure it. He found that the cases which responded to this treatment usually showed an excess of his antiproteolytic factor in their serum, and if untreated went on to the condition of abruptio placentae. (Shute, 1937b.). Young could not confirm these results, and they are too few in number to draw conclusions (Young, 1937.).

In contrast to the cases of toxaemia going on to abruptio placentae, Shute found that those that advanced to eclampsia nearly always showed an excess of prolan, and not of his oestrogenic antiproteolytic factor in their serum. As a result of this, they usually responded better to treatment with oestrin than with vitamin E, as prolan and oestrin have an antagonistic effect, especially on uterine muscle. Still, in one case out of a series of eight eclamptics, he found an excess of his factor in the serum. The finding of these two types of toxaemia caused him to advise care in using either vitamin E or oestrin in their treatment; as, if the serum is not tested for his factor first, the wrong treatment may be given, and the condition become worse. (Shute, 1937c.).

5. Lactation.

Shute gave wheat germ oil to twenty three women in their puerperium to stimulate lactation. This was still defective seven days post-partum, as none of them were producing more than four ounces of milk a day. Fifteen of these women had already been taking the oil during pregnancy, and of these six improved, but the rest did not. Of the remainder, only two showed any increase in the production of milk. If they were going to improve, they experienced a tingling in the breasts five or six hours after taking an ounce of the oil, and the breasts filled in twenty four hours. Treatment had to be continued for two weeks and, if it was stopped, lactation stopped. Vitamin E deficient rats seem to need twice as much of the vitamin for normal lactation as for reproduction, but this did not seem to apply to humans. (Shute, 1938b.).

In another series of cases, Shute reports thirteen mothers out of a total of thirty three, whose defective lactation was rendered adequate by vitamin E. (Shute, 1939.).

It is impossible to draw conclusions from such a small number of cases.

6. Senile Vulvo-Vaginitis.

In some of these cases, Shute was able to demonstrate the presence of his oestrogenic factor in the patients serum, and so considered Vitamin E would be of value in their treatment. (Shute, 1938a.) . This he found to be so if the

wheat germ oil was given in massive doses. Twenty to thirty percent of these cases do not show his factor in their serum, and are made worse by wheat germ oil, but benefited by treatment with oestrogens. He only quotes four cases succesfully treated, but in these all symptoms disappeared, and the affected parts returned to normal. (Shute, 1938a.).

7. The Neo-natal Mortality, and Congenital Abnormalities.

It has been suggested that partial vitamin E deficiency might result in a certain proportion of the human neo-natal mortality. One would expect this, if lack of the vitamin really is a factor in increasing the incidence of premature births, as these raise the infant mortality figures. It has also been noted that mothers receiving wheat germ oil often have unusually large and well-developed babies. For these reasons, it might be suggested that vitamin E be given to all mothers as a prophylactic measure against premature births. (Brown, 1939.).

It has been mentioned before that lack of vitamin E may cause congenital deformities. Shute considers the vitamin might be of use in preventing these, and cites two cases of women who had given birth to abnormal children in their first two pregnancies, but after receiving treatment with wheat germ oil, ~~they~~ both produced a normal child. In one of these cases, he tested the mother's serum during one of her first two pregnancies, when she gave birth to a child with a hare-lip, and found that it contained an excess of his antiproteolytic factor. One of the other woman's pregnancies, before she received treatment with vitamin E, resulted in a mongolian-looking baby; but it seemed intelligent and was apparently normal at twelve months old. She showed signs of abruptio placentae during the sixth month of pregnancy. (Shute, 1938b.).

In another series of cases, Shute noted five cases of congenital abnormalities in children of mothers who showed other symptoms and signs which have been attributed to lack of vitamin E. He also reports that five women were delivered of normal children, after treatment with wheat germ oil, although previously all their children had been mentally defective.

He draws no conclusions from these results, but considers that this is a fertile field for further study. (Shute, 1939.).

8. Dysmenorrhoea.

Shute has also successfully treated patients suffering from dysmenorrhoea with wheat germ oil, when he could demonstrate an excess of his antiproteolytic factor in their serum.

No details of these cases are given.(Shute.1938b.).

A hundred and fifty similar cases were treated with Vitamin E by Juhász-Shaffer, but no improvement was noted. (Juhász-Shaffer.1933.).

Shute also mentions cases of menorrhagia who were improved under treatment with Vitamin E. (Shute.1938a.).

9. Vaginal and Anal Pruritis.

The serum of some patients suffering from anal and vaginal pruritis may apparently contain an excess of Shute's antiproteolytic factor, and if this is the case their symptoms are relieved, accordding to Shute, by treatment with Vitamin E. (Shute.1938b.).

C. The Effect of Vitamin E Deficiency on the Male Reproductive System.

(a) In Experimental Animals.

The Testis.

A detailed description of the effects of vitamin E deficiency on the male reproductive system was first given by Evans and Burr. They noted that rats under these conditions became sterile and that once this was detected it was very difficult to cure it, but they did manage to do so in a fifth of their cases if the vitamin was given on the fifth to the eighth day after the onset. No spontaneous cures were noted. If their rats were weaned and put straight onto a vitamin E deficient diet they became sterile at the close of the fourth or the beginning of the fifth month. (Evans, Burr, 1927.). This has been confirmed by other Experimentors. (Eddy. Daldorf. 1941.), (Juhász - Shaffer. 1933.). When added precautions are taken to deprive the mother of most of her supply of vitamin E as well, these changes will appear earlier than this, even as soon as thirty days after the introduction of the deficient diet, (Mason. Bryon. 1938.). In these latter animals the onset of sterility was noted to coincide with the attainment of sexual maturity (Mason. 1939.). The presence of vitamin Bi seems to retard the onset of signs of vitamin E deficiency in male rats, (Shute. 1939.) and animals deprived of vitamins B and E require longer treatment with a different diet to produce

typical vitamin E deficiency changes. This may be due to a greater conservation of vitamin E than normal, due to a slow growth rate, resulting from lack of vitamin B. (Mason.1933a.).

The sterility was prevented by giving foods rich in vitamin E to the animals but as has been mentioned, once, patho-physiological changes have begun the vitamin apparently cannot stop the degeneration of the affected testicular cells. There may be no structural changes in the testis when the male first becomes sterile, but even if the vitamin is added at this early stage, certain of the cells degenerate and in three-quarters of the animals almost all the tubules may be destroyed. This indicates that some irreversible physiological damage must take place in these cells before they actually show pathological changes, and shows that structural expression cannot be used as an indicator of fatal weakness or reparative potentialities of the testicular tubules. If any signs of tissue repair are observed it must be due to mitotic division of some of the tubule cells, which have escaped damage and are capable of regeneration and differentation to produce normal germinal epithelium. ———————————————————

——— They spread along the dead tubules and give rise to a picture of normal and degenerate tissue mixed intimately together.

The first abnormal findings are a fusion of the spermatazoa in the ejaculate and then they

disappear from the vaginal plug. After that changes begin to occur in the testis itself and if no vitamin E is available the degeneration of the tubules is complete in about two months. The Interstitial tissue is apparently unaffected (Nelson.1933), but if the deficiency is very prolonged there must be some adverse effect as a loss of sex interest takes place, and Ringsted thinks atrophy of this tissue is characteristic of the later stages of vitamin E deficiency in the male animal. (Ringsted.1936).

Evans and Burr noted six stages in the developement of this sterility in male rats. In the first stage the spermatozoa are normal, but the fertilizing power is lost, in the second motility of the spermatozoa is lost, in the third they are fused in groups and invested by Sertoli cells, in the fourth they are absent from the ejaculate, in the fifth the animals lose the ability to form the copulation plug and in the sixth there is a loss of sex interest. The histiological changes in the germinal epithelium are found to start during the first of these stages and advance during the latter ones.

First there is an inhibition of spermatogenic activity with fusion of abnormally staining spermatazoa. Then nuclear chomolysis of the spermatids and secondary spermatocytes takes place and they tend to coalesce to form giant cells. The injured cells may be disolved in situ or transported to the epididymis. Finally with loss of the germinal cells

the seminiferous tubules become shrunken and lined with Sertoli tissue. (Evans Burr.1927).

Mason reported on these changes in more detail and also noted that the testicular changes due to vitamin E deficiency were unique and quite unlike. those due to other causes such as deficiency of vitaman A, inanition or prolonged administration of oestrogens. He based this statement on the irreversable nature of the histiological injuries from an early stage of the deficiency, on the extensive nucleus changes in the germ cells and on the complete removal of the latter from the germinal epithelium. Vitamin A deficiency damages the testis in experimental animals but some of the basal cells persist and are capable of restoring the germinal epithelium when the supply of this vitamin is renewed.

He also noted the nuclear chromolysis and the fusion of the fully mature or maturing sperm, followed by their removal to the epididymis. In the later stages he found that, like the spermatids and secondary spermatocytes, the primary spermatocytes and the spermatogenia fuse together and enter into a similar giant cell formation, although their removal by gradual sloughing is the more usual proceedure.

The giant cells have numerous bead-like or crescent-like nuclei and these changes along with the fusion of the spermatids, and peculiar chromolysis, which takes the form of liquifaction and segregation of the chromatin into beads, are typical of testicular

changes due to vitamin E deficiency. Even when the changes due to vitamin A deficiency are very far advanced a few poorly formed spermatazoa are seen but this is never the case in advanced vitamin E deficiency. Also, in testicular changes due to inanition the germinal epithelium is more or less intact, and soon regenerates when a normal diet is given.

Mason, as well noted a physiological disturbance of the germinal cells which preceeds, and is more fundamental than, the earliest structural changes. He found the changes deep-seated and irreversable and, once they had started, they preceeded in a similar manner and at an identical rate whether vitamin E was at that time present in the diet or not.

He considers that both vitamins A and E are essential for the maintenance of the germinal epithelium but are probably concerned with different phases of their physiological activities. When both the vitamins are lacking the testicular changes appear sooner than when only one of them is lacking. It has already been noted that the stores of vitamin A are lower than normal in states of vitamin E deficiency (Davis Moore.1941.), and Mason suggest that lack of vitamin A may possibly cause a decreased absorption of vitamin E and also alter the metabolic activity of the germ cells, so that they require more of the latter. The changes due to vitamin A deficiency appear at an earlier time than those due to vitamin E deficiency.

If a male animal is deprived of both vitamins A and E, and then supplied only with vitamin A the damage to the spermatids and spermatozoa is greatest while the less mature cells survive longer than in simple vitamin E deficiency, although it cannot be quite certain if the vitamin E deficiency was as great in these experiments as in earlier ones. These findings suggests that vitamin E is more essential for the more complex of the maturation changes than for the early growth changes of the germ cells.

When the lack of the vitamin is only partial and gradual in its onset, only the mature elements of the germinal epithelium are effected and repair of these is sometimes possible if the vitamin is added to the diet at a later date.

A decrease in the weight of the testis of Vitamen E deficient rats has been noted by some Experimentors. (Copping. Korenchevsky.1939.). Biddulph. Meyer.1941.).

All the testicular changes have been confirmed by other Workers. (Kudrjaschov, 1930, 1931.). (Ringsted,1936.). Ringsted reported that the testicular degeneration due to vitamin E deficiency attacks several catagories of the germinal epithelium at a time and does not occur in layers. The intensity of degeneration decreases irregularly from the lumen of the testicular tubules to its basal membrane. He notes no abnormality in the vascular

walls or lumen of these tubules, but describes the formation of so-called'cytological bladders' in the germinal epithelium. These apparently correspond to the giant cells already mentioned. He divides the degeneration into four stages. The first three are concerned with the epithelial destruction and the fourth with the atrophy of the interstitial tissue which this Author considers is typical of the later stages of vitamin E deficiency. (Ringsted.1936.). Vogt-Möller suggests that bulls may be responsible for cases of abortion in cows owing to morphological defects in their spermatozoa. These defects are referable to pathological changes in the epithelium of the seminiferous tubules, and are similar to those seen in vitamin E deficient rats. (Lagerlöf. 1934.). So he treated six bulls, showing this testicular transformation, with vitamin E but with apparently no effect on the spermatozoa although one of the bulls became fertile. (Vogt-Möller. 1939.). Degeneration of the testes of vitamin E deficient male mice has not been noted. (Mason .1939.).

It may also be recorded here that the doses of vitamin E needed to cure changes in the testicles of male animals are larger than those needed to cure pathological conditions in the female animal. This is what would be expected from the fact that the damage in the male is mainly to its own tissues and is irreversable while in the female it is the embryo which is chiefly affected. (Mattill.1938.).

However this higher requirement for vitamin E of seminiferous epithelium, in proportion to its mass, is difficult to correlate with the rate of cell division or protoplasmic growth as both these are greater in the embryo. (Evans, 1932).

In contrast to the requirements of the curative dose, Mason found that very small doses of vitamin E prevented the onset of testicular degeneration in vitamin E deficient rats, or at least delayed its onset for some time; but similar doses did not prevent resorption during the first pregnancy if fed to rats newly weaned by vitamin E deficient mothers. The prophylatic requirements of the male were about a tenth those of the female, and he suggests this may be due to sex differences in absorption, storage or utilization of the vitamin. (Mason, 1939). These findings are difficult to correlate with the former ones.

In female vitamin-E-deficient animals the ovaries rarely show any abnormal changes. This difference in the sexes is possibly explained by the fact that both the ovary and testis start with an equal store of vitamin E, but when this is removed from the diet, the testis by its rapid cell division soon disipates its store while the ovary conserves its, and so can remain functionally normal for a longer period. By feeding vitamin E deficient animals with normal testis it has been found that little of the vitamin can be stored in it, as it does

not cure the deficient animals. (Mason.1933a.). The theory that there might be two specific forms of Vitamin E for the two sexes has not been confirmed. (Mattill.1938.).

The Pituitary.

In some cases of vitamin E deficient male rats examination of the pituitary has revealed changes not unlike those that occur after castration. They usually do not appear till the animal has been four months on a deficient diet. There is an increase in the size and number of the basophil cells but they do not attain the 'signet-ring' appearance which is typical of change occuring in these cells after castration. Nelson states that these changes indicate a storage of the gonad - stimulating hormone. Degeneration of the acidophil cells has also been described in these glands. (Severinghous. Smelser. 1933). The rate of the development of these changes depends on the amount of the vitamin in the basal diet and in the individual susceptability of the animal to vitamin E deficiency. Once they have started vitamin E has no curative value. (Nelson 1933b). These Pituitary glands from vitamin E deficient animals are apparently increased in weight after three to six months of such deficiency, but after twelve months this increase disappears. (Biddulph. Meyer, 1941).

Nelson also noted that these glands contained more gonad - stimulating hormone than

normal, when extracts of them were tested on the ovaries of immature hypophysectomised and normal rats, although the increase was not so marked as in the pituitories of castrated rats. (Nelson.1933b.). Other Experimentors have confirmed this result. (Engel.1929.). (Drummond. Noble. Wright. 1939.). (McQueen Williams. 1934). However, some found no significant difference in their gonadotropic potency.

The Accesory Sex Glands.

These glands are influenced by hormones produced by the interstitial tissue of the testis, and as this tissue is apparently little affected in vitamin E deficient animals, one would not expect to find them significantly altered by lack of the vitamin.

Some Experimentors have found them unchanged. (Evans. Burr. 1927), but Nelson noted that there was an inability to form the vaginal plug in vitamin E deficient rats which indicates some disturbance of the glands. They showed slight signs of degeneration after a hundred and forty five days of vitamin E deficiency and this suggest that the production of sex hormones by the interstitial tissue of the testis is finally impaired in the absence of the germinal epithelium. (Nelson.1933b.). The occurence of this degeneration of the seminal vesicles and the prostrate has been confirmed by other Experimentors. (Evans. 1932.). An increase in weight of these glands, especially of the prostrate has been described in

states of vitamin E deficiency in animals and can be correlated with the finding of an increased gonadotropic potency of the pituitaries of the same animals. (Copping. Korenchevskey. 1939.). According to Biddulph and Meyer this increase in weight only lasts for three or four months and after about fifteen months of vitamin E deficiency there is a decrease in their weight. (Biddulph. Meyer. 1941.). The seminal vesicles of these animals in certain cases were found to be discoloured due to deposition of small yellow granules in the cells of this organ. (Moore. Martin. Rajagopal. 1939.).

Mason suggest that really the glands are unaffected by lack of vitamin E and that the degeneration observed was due to a constitutional inferiority, due to the experimental diet. (Mason. 1939a.).

The Adrenals, The Thyroid and The Thymus.

The Adrenals of vitamin E deficient male animals have been found to be increased in weight. A similar change has been found in the thyroid of these animals, and involution of their Thymuses is delayed for longer than the normal time.

Sexual Behaviour

Abnormalities of sexual behaviour have been recorded in cases of male rats fed on a diet deficient in vitamin E. However it is not certain if these were due to lack of the vitamin, as they continued when it was added to the diet. (Bacharach.

Wiesner. 1937.).

Some Experimentors found that in the later stages of vitamin E deficiency in the male animal there was a loss of sex interest. The same Experimentor usually demonstrated atrophy of the interstitial tissue of the testes of these animals. (Evans. Burr. 1927.). (Ringsted.1936.). A similar loss of sex interest in vitamin B deficiency has been noted. (Evans.1932.).

(b). In Human Beings.

The Testis.

Mason examined testes from men who had died suddenly from accidental causes. In some cases he found a large number of multinucleated giant cells derived from the spermatids and resembling those seen in the testes of vitamin E deficient animals. He suggests these changes might be due to a mild degree of vitamin E deficiency but admits that the evidence is too inconclusive to draw conclusions from. (Mason.1939a.). A similar histological picture in apparently normal testes has been described by other Authors. (Di. Biasi.1930.).

The lack of evidence of pathological conditions occuring in the male due to deficiency of vitamin E, as compared with the many conditions, which are suggested to be due to its deficiency in the female, may be due to the very much smaller dose of the vitamin which is apparently needed to prevent changes in the male as opposed to the female.

(Mason. 1939b.).

Shute detected his antiproteolytic factor, which he states is present in cases of vitamin E deficiency, in the serum of fifty to seventy per cent of normal males during the spring months. (Shute.1938 b.).

(c) Theories of the Mode of Production of the Effects of Vitamin E Deficiency in Man and in Animals.

The Cytological Theories.

If vitamin E is needed for normal cell division it is obvious that the testis, which is an area of rapid cell proliferation, would be affected before almost any other tissue of the male body. This theory has already been discussed.

Mason suggests that vitamin E is essential for the more complex maturation changes of the cells of the germinal epithelium and that lack of it interferes directly with the normal physiological processes of these cells. He offers no explanation of this phenomena. (Mason. 1933a.).

Pituitary Dysfunction and Disturbances of the Testicular Hormones.

The possibility has been raised that the effects of vitamin E defiency in the male are due to anterior pituitary abnormalities. Experimental evidence does not support this theory. They may also be due to a disturbance of the production of the testicular hormones.

The gonads are stimulated by the pituitary, but the gonadal hormones have an inhibitory influence on the latter. If the pituitary is removed the gonads atrophy, but removal of the gonads permits abnormal pituitary activity and this latter change has been noted in states of vitamin E deficiency. This seems to indicate that it is the gonads, not the pituitary which are primarily affected.

This is supported by the histological findings in pituitaries from vitamin E deficient animals which resemble those seen after castration. (Wagenen.1925). However it has been noted that the interstitial tissue of the testes of these animals is usually unaffected and it is this tissue which produces the gonadal hormones. It has been suggested that there are two testicular hormones, one produced by the interstitial tissues which control the necessary sex glands and is unaffected by vitamin E deficiency and one produced by the germinal epithelium which controls the pituitary and is reduced in quantity or abolished by this deficiency. (Matham. Cramer.1923). (Nelson.1933b.). A more likely explanation is that the total quantity of gonadal hormone produced by the interstitial tissue is reduced by degeneration of the germinal epithelium. This results in there being enough to preserve the accessory sex glands but not enough to control the pituitary. This is supported by the experiment of giving graduated doses of testicular hormone to castrated animals with the

result that a dose is reached which will maintain the cells of the seminal vesicles and prestate in a normal state but not those of the pituitary. If the dose if further increased the pituitary returns to normal. (Nelson.1933b.). In view of this explanation it is possible that these experimentors who found no increase in the gonad - stimulating hormones in the pituitaries of vitamin E deficient male animals may have been using ones in which the testicular degeneration was not far advanced enough to influence the output of gonadal hormones. So the testicular hormones are also apparently secondarily involved, probably by the effects of lack of the vitamin on other testicular tissues, and their disturbance is not an etiological cause of the changes seen in vitamin E deficient male animals.

The damage to the testis resulting from hypophysectomy is unlike that due to vitamin E deficiency, being more like that seen in cases of inanition, as both tubules and interstitial tissue atrophy, and injections of pituitary extracts containing gonadtropic hormones have no effect on the testicular degeneration of either vitamin A or E deficiency. These facts also suggest that primary pituitary damage due to lack of vitamin E is not a casual factor of the testicular changes. (Mason.1933a.). (Evans.1932.). (Drummond.1939a.). (Giller.1934.).

The fact that the interstitial tissues of the testis are so little affected by vitamin E

deficiency is certainly against lack of the vitamin inducing a grave disturbance of the gonad-hypophyseal relationship in the male. It is also unlikely that the effects of the deficiency are produced by a disturbance of this sort, as in vitamin E deficient males, pituitary extracts only stimulate the interstitial tissue and do not affect the degenerated tubules, while hypophysectomy only further decreases the weight of the testis. (Drummond. Noble. Wright.1939.).

Shute's theory of vitamin E deficiency resulting in the unapposed action in the body of oestrogens has already been discussed. It has been stated that the testicular changes of vitamin E deficiency are unlike those resulting from continuous injections of oestrin . (Drummond.1939c.). Shute has argued that injections of oestrogens may produce different effects to those that these substances produce, when naturally circulating in the body. He also states that sudden injections of oestrogens may call into play a compensatory mechanism, while in states of vitamin E deficiency, where the excess of oestrin has been continuous, this mechanism may break down, so that they act unapposed and produce different results to those of the injected oestrogens. (Shute.1940a.).

No testicular regeneration has been noted following injections of either testicular or ovarian hormones. (Drummond.1939a.). This argues against a direct relationship between the vitamin and these

hormones. However, Adamstone suggested that vitamin E was needed for the effective ~~use~~ utilisation of andogens. He found that castrated birds receiving both testosterone and Tocopheral responded much better to treatment than if they were receiving the testosterone alone. (Adamstone, 1941d.).

The results of therapy give some support for a relationship between vitamin E and the gonadotropic hormones. Similar results have been noted on treating undescended testes with either of these two substances and it is known for certain the gonadtropic hormones are necessary for the normal descent of these organs. (Clark, 1940).

Finally it has been suggested that the testicular degeneration may have to be considered as a neurogenic phenomenon. Lack of vitamin E apparently results in degeneration of the systemic nervous system and if the sympathetic nervous system was likewise affected, changes in the testis would undoubtedly occur. (Einarson. Ringsted. 1938.).

The evidence in support of all these various theories seems to indicate that the most likely explanation of the effects of vitamin E deficiency is a fundamental disturbance in the normal functioning of the testicular cells. The pituitary is apparently secondarily affected and there is little evidence of the vitamin being primarily associated with the testicular hormones, unless lack of it damages the cells which are responsible for its production.

The possibility that Shute's theory, of the testicular changes being produced by the unapposed action of oestrogens, being the rightful explanation warrants further investigation.

(d) The Application of Vitamin E to Clinical Medicine.

1. Sterility.

Moench used vitamin E in the treatment of sterility in the male with apparent success. He gives no details of the cases. (Moench 1936).

Shute reports one case of a man who was impotent and showed gross testicular atrophy. Under treatment with wheat germ oil he became once more potent and the testis grew markedly, but retrogressed when the treatment was stopped on three seperate occasions. (Shute.1938b. 1939.).

However Shute's general conclusions are that vitamin E is of no value in the treatment of testicular hypofunction or of the resulting conditions of azoospermia or neerospermia. (Shute.1939.).

2. Undescended Testicle and Genital Hypoplasia.

Successes, equal to those of gonadotropic hormones, have been claimed for vitamin E in the treatment of undescended testes in boys and of underdevelopment of the external genitals and secondary sexual characters in older male patients. Successful results were sometimes recorded after use of the vitamin when the hormones had had no effect. (Clark.1940.). (Shute.1938b.).

3. Prostatic Hypertrophy.

Shute has suggested that, as vitamin E according to his theory counteracts the effects of the oestrogens, it might be of value in any condition associated with an excess of these substances. He gives prostatic hypertrophy as an example of such a condition but has not apparently tried to treat it with vitamin E. (Shute.1939.).

D. The Effect of Vitamin E Deficiency on the Nervous and Muscular Systems.

(a). In Experimental Animals.

The question has been raised whether the lesions formed in the neuro-muscular system of vitamin E deficient animals are really due to lack of the same principle that causes the reproductive changes.

It was found that wheat germ oil, treated with ferric chloride to destroy the vitamin E, could prevent the development of muscular dystrophy in vitamin E deficient rats but not the occurance of abortion. This does not necessarily mean that there are two different factors, but probably in the female, the reproductive system needs more of the vitamin than the neuro-muscular system, or young rats may differ in their ability to utilise possible precursors for the synthesis of vitamin E. It is known that ferric chloride does not completely destroy vitamin E. (Geottoch. Ritzmann.1938.).

Muscular changes are sometimes seen to appear

in the male animal before testicular ones, when they are deprived of vitamin E: , which suggests that diets which produce the former may contain sufficient of the vitamin to prevent the latter changes. (Mackenzie. McCallum. 1941). However, other Observers have found that the sterility in males also results before the paralysis and have suggested that both may be due to one principle; ~~but~~ and that the paresis is more slow to appear and more difficult to observe at first, or to two different principles, with differences in the requirements of them, in the size of their storage depots in the body and the amounts present in the food. They noted that the neurotropic factor had similar actions to vitamin E in the treatment of sterility and also protected the vitamin E deficient animal against the onset of paresis, although it did not cure it once it had started. They admit that as they used crude preparations of the vitamin and that, although the latter action is due to the lack of a nutritional principle with neurotropic function important to the preservation of the anatomical integrity of certain parts of the central nervous system, it is not certain if this is absolutely similar to the factor essential for reproduction. The fact that the vitamin E does not cure the paresis once it is established does not disprove that the etiological factor is vitamin E deficiency, as an irreversible change may take place in the neuro-muscular system. (Einarson. Ringsted.1938).

Other Experimentors have also suggested there are two different principles in wheat germ oil, one for the reproductive system which has been termed vitamin E and one for the nervous system. (Goettsch. Poppenhiemer. 1931.). (Morgulis. Spencer. 1936.). (Morgulis. Wilder. Eppstein. 1938.).

From more recent experiments this seems to be untrue, anyhow for most animals, and most types of muscular lesion produced by vitamin E deficiency. α Tocopherol is undoubtedly the factor needed for normal reproduction, and muscular dystrophies in vitamin E deficient animals have been cured and prevented by this substance. (Goettsch. Ritzmann. 1939.). (Evans. Emerson. 1940.). (Mackenzie. Levine. McCollum. 1940.). Finally it may be noted that the paralytic synpdromes produced by lack of other vitamins such as vitamins A, B and C can easily be distinguished from those produced by deficiency of vitamin E, and unlike the latter are accompanied by profound constitutional changes and almost always end fatally. Vitamins A, B, C and D have no effect on the muscular dystrophies of vitamin E deficient animals. (Drought. Shaffer. 1941.).

It has also been suggested that there are two neurotropic factors. It is possible that there may be a water-soluble factor in wheat germ oil, which may prohibit autoxidation and destruction of vitamin E in the oil, when it is added to a rancid diet used in the production of the dystrophies in animals. However it is unlikely that there are two such factors necessary for

the normal function of the neuro-muscular systems alone. This problem will be considered again later.

Many Experimentors have demonstrated lesions in the muscles of vitamin E deficient animals but few have discovered lesions in the nervous systems of these animals. For this reason a description of the muscular lesions found will be given first.

Muscular Dystrophy.

Muscular changes occur when various animals are deprived of vitamin E. As the changes differ in small particulars from animal to animal, with nervous symptoms predominating in one and muscular in another, the pathological conditions found in each of the animals will be considered in turn. The rat will be dealt with first, as this is the experimental animal most often used, and certain general conditions, which apply to all the animals, only considered in relation to this one. It may also be noted that even in the same animal the muscular lesions may differ, depending on the extent of the deficiency, the age and sex of the animal, and whether there is a lack of other vitamins as well as of vitamin E. It has been suggested that, in congenital cases when the offsprings are deprived of their supply of vitamin E through the placenta and mammary gland, owing to such causes as defects in maternal absorption, different grades of muscular involvement may result corresponding to the severity of the deficiency. In mild cases there is just a muscular

hypotonia with poorly developed muscles, retarded growth and slowness in beginning to walk; but in severe cases myotonias result and these may also vary in type from hypertrophic to atrophic. Improvement in these cases without treatment may be due to improvement in the diet. It is possible these suggestions apply to humans as well as to animals. (Stone. Manchester. 1941.).

If the paralysis in rats is well established it has been found by various Experimentors that it is not cured by vitamin E and the value of this vitamin seems to be in the prophylaxis and possibly the early treatment of these dystrophies. (Marchesi.1935.). (Burr. Brown. Mosely. 1937.). Young rats showing the signs of muscular dystrophy apparently respond to treatment with α Tocopherol for a longer time than old ones. (Morris.1939.). The onset of the dystrophy and death is prevented in the offsprings of vitamin E deficient rats if the vitamin is given before the fifteenth day of life, (Evans.1938.), or if it is added to the mother's diet up to ten days after mating. (Morelle.1931.). Some Authors state that this paralysis is ~~due~~ solely due to muscular lesions, but in view of the findings of Einarson and Ringsted, to be recorded later, it is difficult to see how this can be so. (Olcott.1937.), (Chlor - Dolhart.1939.), (Geottsch. Poppenhiemer.1931.), (Geottsch. Ritzmann. 1939.).

Details of the experiments carried out on

vitamin E deficient rats will now be given. Evans and Burr were the first to note paralysis in the young of vitamin E deficient rats. Its onset was on the nineteenth to the twenty-fifth day, which is much earlier than the onset of the muscular dystrophies described in the following pages. This may be due to a severer deprivation of the vitamin or the paralysis may be of a different type/ to that occuring on depriving rats of vitamin E after they have been weaned. It was in fact a spastic paralysis indicating involvement of the central nervous system, so it will not be discussed in detail till a later section. It was prevented by giving the vitamin before the symptons appeared but never cured once this had happened. (Evans.Burr.1928.). Blumberg was the first to note changes apparently confined to the muscles of these animals and also found they showed signs of general malnutrition and retarded growth. (Blumberg. 1935. 1937.). Knowlton and Hines put rats born from, and weaned by, mothers partially deficient in vitamin E, on a diet completely deficient in this vitamin. After four to six months the tension developed by the muscles was below normal and the tension in response to nerve stimulation was below that resulting from direct stimulation. The Creatin content of the muscle was lower and the chloride content higher than normal but this problem will be considered later. On microscopic examination some focal necrosis and proliferation of the sarcolemma nuclei was found and the picture was not unlike that seen in certain types

of human myopathy when the clinician can show marked impairement of function although the pathological changes found are slight. The onset of the functional and chemical evidence of muscular dystrophy after five months on a deficient diet agrees with the findings of some Authors, but not of others, who found that it might take as much as twenty-two months before changes were found. (Burr. Brown. Moseley, 1937). In these rats there was a slight response to the intravenous injection of acetyl choline bromide, and there is no response with similar injections given to atropinised eserinized rats, and this seems to indicate the peripheral motor neurone is only slightly affected as denervation sensitises muscles to acetylcholine. Finally it was noted that quantitatively the changes were less marked in female rats, but whether this sex difference was due to the gonadal or somatic effect of the deficiency is not known. Wheat germ oil protects the young rats from these muscular lesions if given ten to twenty-five days after birth. (Knowlton. Hines. 1938).

Evans, Emerson and Telford put twenty-one day-old rats, after weaning, on a vitamin E deficient diet and carried out autopsies after twenty-two months. Before death the rats were irritable, breathed abnormally fast and showed marked atrophy of their muscles. On microscopical examination of these muscles an infiltration of leukocytes and connective tissue elements was found, the cross striations of

the skeletal muscles were reduced and the sarcolemma neiclei were pyknotic. Within the sarcolemma there were numerous nuclei arranged in rows parallel to the long axis of the fibres and these might persist after the fibre was resorbed. The fibres were often replaced by fat and connective tissue and in some cases both the longlitudinal and cross striations were absent. The first stages of the muscular degeneration were often demonstrated microscopically before there were any obvious signs of paralysis. (Evans. Emerson. Telford. 1938.). The same Authors give a more detailed description of these changes in which they state that the lesions first manifest themselves in waxy, hyaline or Zenker's degeneration of individual muscle fibres. Then the contractile substance of the fibre necroses and breaks up into swollen hamogenious hyaline segments. These masses attain double the diameter of normal fibres, but eventually the segments loose their hyalinisation, become granular, reduced in size and finally can only be identified as debris in the intersarcolemma spaces. The nuclear changes are the same as described above. There are many areas of normal muscle mixed with the areas of degeneration. The gastrocnemius and steno-mastoid were the muscles most often affected and the masseter and tongue muscles least. (Telford, Emerson. Evans. 1939. 1940.).

These findings of muscular changes coming on twenty two months after starting to feed rats on a Vitamin E deficient have been confirmed. There

seems to be no doubt that they are due to lack of vitamin E although they are not cured except in their early stages, by this vitamin or by vitamin B. (Burr. Brown. Moseley. 1937.). Morris gave synthetic α tocopheral in doses of 17, 18, 20, 26, 51, and 56 milligrams to rats deficient in vitamin E and suffering from muscular dystrophies. All the animals except those receiving doses of seventeen milligrams recovered. This seemed to indicate that twenty milligrams is near the lower limit of the single curative dose in these conditions, and also that they must be due to a deficiency of α tocopheral and no other substance. He never observed any cases of spontaneous cure. (Morris. 1939.).

It may be noted, in view of the attempts to cure muscular dystrophies in man with vitamin B in combination with vitamin E, that it has been reported that massive doses of vitamin Bi cure cases of similar dystrophies in vitamin E deficient rats. The rats were deprived of the vitamin from birth and they were about twenty two days old when they first showed muscular lesions. The response to the dose varied with the severity of the dystropy but an average of four to fourteen internatial units cured each rat. (Holmes. Pigott. 1941.). This work has not been confirmed.

Also muscular weakness, associated with degenerative foci in the muscles has been described in rats deprived of vitamin B6. Acrodynia, a pathological condition of the skin, may overshadow the

muscular lesions in these animals. Massive doses of vitamin B6 given to rats result in muscular tremors convulsions and marked impairiment of the righting reflex. (Antopol, Unna, 1940.). It has been suggested that vitamin B may require the co-existence of vitamin E for its proper function as there might need to be a balance between the two. (Einarson. Ringsted. 1938.).

During his experiments on the changes found in the pituitary and thyroids of vitamin E deficient rats Barrie noted no microscopical lesions in their central nervous system on examination with the Weigart Pal and Marchi techniques, but females partially deficient in vitamin E were unable to rear their litters, and the latter grew more slowly, finally showing about the eighteenth day after birth, a flaccid paralysis of the limbs with spasms of the feet. Most of them died. (Barrie, 1937a.). He cured this paralysis by crude preparations of the vitamin and also by synthetic preparations. In the latter case he used two milligrams of synthetic d - l-α-tocopherol. This leads him to believe that the missing factor in producing these changes is vitamin E and that there are not separate factors from the reproductive and nervous systems. (Barrie, 1938a.).

These findings suggest that a primary muscular dystrophy occurs in vitamin E deficient rats although the central nervous system has not been examined very carefully in some cases. They are

confirmed by Olcott who also suggests that the dystrophy may be present in a milder form in animals which show no external symptoms of paralysis or weakness, and that vitamin E given at this stage prior to their onset, if it does not prevent it, alleviates the severity of the dystrophy. (Olcott. 1937.). Other Experimentors have cured and prevented the muscular dystrophy with synthetic vitamin E. Knowlton and Hines injected three milligrams of synthetic α tocopheral acetate subcutaneously every week for one month and cured the condition. ~~He~~ They noted that muscular changes occured before the onset of symptons in these vitamin E deficient animals. There was an increase in the concentration of the water and chloride in the muscle and a decrease in the maximum strength and the creatine concentration. Focal hyaline necrosis was also demonstrated, beginning after one month and being well defined after two months of vitamin E deficiency. (Knowlton. Hines. Brinkhous. 1939.), (Demole. Pfaltz. 1939.), (Evans. 1940.). Martin and Moore consider that the degeneration and necrosis of the skeletal muscles may be related to the brown discolouration found in the ~~u=teri~~ of vitamin E deficient female rats. (Martin. Moore ~~1936.~~). Mackenzie noted the occurance of a flaccid paralysis of the legs of vitamin E deficient rats followed by tremors and convulsions of the forelegs and head. The paralysis was arrested but not cured by vitamin E and growth was started again.

(Mackenzie. Mackenzie. McCollum. 1940.). Morgulis found muscular dystrophy, as opposed to nervous changes, was more common in younger rats and there was a greater regeneration under treatment in these animals. (Morgulis. Wilder. Eppstein. 1938.).

The occurance of muscular dystrophy in vitamin E deficient rats has been described by many other Authors. (Madsen.1936.), (Morgulis. Spencer. 1936.), (Morgulis. Spencer. Wilder. 1938.), (Pappenhiemer.1939.).

Next, similar lesions found in vitamin E deficient rabbits will be described. Goettsch and Pappenhiemer fed rabbits and guinea pigs on a diet treated with ferric chloride to destroy the vitamin E content and they developed muscular dystrophy. Rats treated in a similar manner were unaffected. The addition of vitamin E to the diet did not prevent it. It was not caused by starvation, infection or scurvy and although it appears unlikely from these results, further experiments have shown deficiency of vitamin E to be the causal factor. After the animals had reached a weight of four hundred grams growth stopped, they became generally flabby and had difficulty in righting themselves if they were placed on their backs. Their weight remained constant for one month and then rapidly declined, the animals dying suddenly two or three days later. Oestrus was normal but they did not live long enough for studies on reproduction to be carried out on them. Male rats at autopsy had

normal sperm. The muscles were atrophic with a yellowish colour and were less translucent than normal. Some were gritty and streaked as though calcification or infiltration with fat had occured. They failed to contract when severed and lost their irritability to mechanical stimulation. The body fat was normal, as were the internal organs except for some fatty infiltration. Microscopic examination showed a coagulative necrosis of the muscle fibres, which showed a typical waxy, hyaline or Zenker's degeneration. This is followed by a reactive cellular multiplication which may fill the sarcolemma sheath with attempts at muscular regeneration. Some fibres were resorped and replaced by fat and connective tissue. The final stage is similar to that described in the case of vitamin E deficient rats and is not unlike that seen in pseudo-hypertrophic muscular dystrophy in man. The smooth muscle is unaffected and no change was found in the spinal chord or the peripheral nerves. Experiments were also carried out to show that the lesions were not due to starvation, infection, scurvy or lack of vitamins A. B. D and G. Goettsch suggests that a multiple deficiency is at work but similar dystrophies have been prevented by α tocopheral alone so this is unlikely. (Goettsch. Papphiemer.1931. 1934). The same Authors describe a muscular dystrophy occuring in the offsprings of vitamin E deficient rabbits while still in utero. The mothers might also develope a similar

dystrophy. In the same animals they noted a degeneration of the epithelium of the convaluted tubules of the kidney without inflammation of the interstitial tissue or the glomeruli, and with prolonged deficiency, skin sores, emaciation and death occured. These changes were prevented by vitamin E. (Goettsch. Pappenhiemer.1936).

Mackenzie demonstrated a nutritional muscular dystrophy in rabbits resulting from deficiency of a fat soluble factor and cured it by administration of α tocopherol. Continued administration of this substance prevented recurrance of symptoms even in acute cases. He noted that treating the diet with ferric chloride only destroys ninety three percent of the anti-dystrophic factor in the unsaponifiable fraction of wheat germ oil, so that if this method is used allowances must be made for the undestroyed part. (MacKenzie, McCallum.1940).

Morgulis and Spencer state that they found a primary muscular dystrophy in dieted rabbits which could not be cured by dried alfalfa grass, vegetable oils, lettuce or vitamins A. B or E if they were given singly. The diet of the animals was treated with ferric chloride to destroy the vitamin E but neither omission of the ethereal ferric chloride treatment or substitution of aqueous ferric chloride treatment entirely abolished its dystrophy producing effects. The dystrophy was prevented and cured by feeding the animals with the diet treated with ferric chloride and in addition fresh green alfalfa grass, lettuce and vitamin E, or dried alfalfa grass and vitamin E, or whole wheat germ. It is suggested that there may be two factors in the cause of the dystrophy, both present in fresh green alfalfa or whole wheat germ and one present in wheat germ oil and the other in dried alfalfa grass. One of the factors is fat soluble, and is easily destroyed by ethereal ferric chloride, or by drying or extraction with water or alcohol, and is probably vitamin E. Its distribution is the same as the latter. The other is water soluble and can be extracted with alcohol and may be a member of the vitamin B complex, possibly vitamin B4. Vitamins Bi, B2 and B6 have been excluded by experiments on rabbits. It is present in yeast, wheat germ, ~~and~~ lettuce, and vitamin B concentrates as the dystrophy is prevented by giving these substances along with vitamin E. These results are not conclusive and may have been due to some technical fault in the experiment such as the incomplete destruction of the vitamin E by the ferric chloride. It has already been mentioned that it is unlikely that two factors are operative in producing the dystrophy, as the cure of muscular dystrophy in experimental animals, on a diet deficient in both vitamin E and the water soluble factors, by synthetic vitamin E rules out the possibility of vitamin B being present in the same dose as the vitamin E when crude preparations were used. The fact that cures of this nature have been recorded makes the

advantage of crude preparation over synthetic ones for the treatment of nervous diseases in man, doubtful. (Columbie. Mattill.1940.). (Demole.1940.). A flaccid paralysis, unconnected with the muscular dystrophy, was noted in some of the rabbits which suggests that a primary lesion of the nervous system may exist in conjunction with a primary muscular dystrophy. They may have a common cause but this will be discussed in the next section. Rats appear to be unaffected for a long time by being fed with a diet such as was used in these experiments to produce muscular dystrophy in rabbits. (Morgulis. Spencer. 1936.). (Morgulis. Wilder. Eppstein. 1938.).

Muscular Dystrophy in vitamin E deficient rabbits, whether young or old, has been cured by synthetic α tocopheral by various Authors. These results are important, as not only does it suggest that the dystrophy is caused by one factor and that the same as is necessary for the function of the reproductive system, but it enables large doses to be given in these conditions which is impossible when using crude wheat germ oil. (Mackenzie. McCollum.1939.), (Morris.1939.), (Demole.1940.).

Finally Mackenzie and McCollum found that on giving twenty milligrams of α tocopheral to vitamin E deficient rabbits with muscular dystrophy, there was a fall of urinary creatine. This question of creatine excretion in cases of muscular dystrophy will be considered later but this finding is a definite proof that the vitamin E controls the dystrophy. They also found that massive doses of the α tocopheral cured the dystrophy in some cases and in others, extended life and promoted growth for some months, although it did not cure the condition. (Mackenzie. McCollum.1941.).

The occurance of muscular dystrophies in vitamin E deficient guinea pigs has already been noted and described as the lesions were exactly similar to those seen in rabbits. (Goettsch. Pappenheimer.1931.). These findings have been confirmed and (prevented and cured by vitamin E. (Wood. Hines.1937.). The muscular dystrophy lesions are very like those occuring in man in cases of juvenile muscular dystrophies. The general metabolism of the guinea pigs as well as their muscles seems to be affected by lack of vitamin E.

The dystrophy in these animals has also been cured by α tocopheral in doses of 1.5 milligrams as well as by wheat germ or wheat germ oil. The muscular creatine was maintained within normal limits for as long as this treatment was continued. (Shimotori. Emerson. Evans.).

Mice, on a vitamin E deficient diet, did not seem to develope testicular degeneration but necrosis of their

muscles has been demonstrated. Twenty per cent of the offsprings of vitamin E deficient mothers developed muscular dystrophy. If they were killed on the first day oedema of the subcutaneous and intramuscular tissues was seen and a third of the cases showed hyaline necrosis of the muscular fibres. The incidence of muscular lesions was highest between the sixteenth and thirty fifth days of life, on an average fifty nine percent of the animals being involved during this time. Early calcification of the necrotic fibres and signs of active regeneration were also seen. In the adult mice scattered hyaline and calcified fibres were demonstrated showing evidence of earlier lesions, but no progressive dystrophy of the muscles or lesions in the central nervous system were found, even though they were still deprived of the vitamin.

Muscular Dystrophies have been produced in ducklings by depriving them of vitamin E and this has been prevented by giving two milligrams of d_l - α tocopherol daily for nine days and then four milligrams daily for the rest of the experiment. (Pappenhiemer. 1940). Ataxia and muscular tremors have also been demonstrated in growing chicks and ducks on a vitamin E deficient diet, although these changes are more likely due to an encephalomalacia or spinal lesion than to a primary muscular dystrophy. (Pappenhiemer. Goettsch. Jugherr. 1939).

A similar condition comes on in dogs after about twenty days on the vitamin E deficient diet, and is cured by synthetic α tocopherol if the treatment is initiated before the symptoms are too far advanced. (Anderson. Elvehjem. Gonce. 1939.), (Brinkhous. Warner. 1941). Puppies also develop a muscular dystrophy if deprived of the vitamin and this is also curable in the early stages. (Morris. 1939.). Although it has been stated that goats do not need vitamin E for their normal body functions, a muscular dystrophy with necrosis of the muscle fibres has been noted in these animals deprived of the vitamin, and also in sheep under similar conditions. (Madsen, McCoy. Maynard. 1933.). Herbivora have often been found to develop a muscular dystrophy if cod-liver-oil was included in the diet. This substance was at first thought to be toxic, but a more likely explanation is that the food remains a long time in the caecum of herbivora and during that time the vitamin E in the diet may be oxidised and destroyed by the cod-liver-oil. (Mattill. 1939.). Turkeys, deprived of vitamin E, show a patchy hyaline necrosis of the smooth muscle of the gizzard although the central nervous system and the skeletal muscles escape damage. (Pappenhiemer. 1940.). Finally a muscular dystrophy has been described in tree-kangaroos if they are fed on a vitamin E-free diet. (Goss. 1940.).

Creatine and Creatinine Metabolism.

Before continuing to the description of the lesions of the central nervous system a few facts about the metabolism of Creatine and Creatinine, and the effect of muscular dystrophy and vitamin E on this, may be mentioned. This problem will be mentioned again when the muscular lesions in human beings, possibly due to lack of vitamin E, are discussed, as estimations of the daily output of ~~the~~ Creatine and Creatinine are a valuable estimation of the effectiveness or otherwise of the treatment of these conditions.

In experimental animals the amount of creatine in the urine increases when they develop a muscular dystrophy, and this may preceed the gross symptoms by as much as two weeks or more. The increased excretion of creatine is not apparently due to loss of weight or starvation as a marked reduction in the urinary creatine occurs within twenty four to forty eight hours of administering vitamin E. No comparable change was noted to occur in the creatinine metabolism of these animals. (Mackenzie, McCollum. 1940.). However the same Authors state from further experimental data that, although vitamin E usually results in a fall of urinary creatine in dystrophic rabbits, sometimes the creatine remains at a high level in spite of the administration of the vitamin, and the creatinine content of the urine falls on the seventh day of treatment. It is possible that in the earlier experiments the animals died before this could occur. (Mackenzie. McCollum. 1941.).

A decrease of the normal creatine content of the muscles of dystrophic rats and rabbits has also been described. (Goettsch. Brown. 1932.), (Knowlton. Hines. 1938.).

It has been suggested that α tocopheral is concerned with the fixation of creatine in the body but no explanation is given to explain this action.

The Central Nervous System.

It has already been stated that Evans was the first to describe cases of paralysis in vitamin E deficient animals and although he did not demonstrate lesions in the central nervous system the paralysis was usually of the spastic type, indicating that these were probably present, and for that reason the details of his experiments will be given in this section.

He noted that female rats, who were sterile, ~~and~~ when given small doses of vitamin E at the beginning of gestation, or put on a diet of casein, corn starch, lard, salts and vitamins A and D, eventually became sterile again, but

were able to produce at least one litter first. Laction was interfered with and growth of the young impaired. This growth was restored with vitamin E, but a day or two before weaning, on the twenty first day of life, they suddenly developed a paralysis. They had difficulty in regaining their limbs if put on their backs. By the twenty first day most of the rats showed paralysis of part of the muscular body wall and of the posterior limbs, and by the twenty fifth day almost all of them showed it. In nearly every case the paralysis was spastic in type but a few of the animals showed an initial flaccid type. It increases in severity if no vitamin E is given and thirty five per cent of the animals die in convulsions. Forty eight per cent show signs of paralysis for the rest of their lives and seventeen per cent recover spontaneously. Death was not due to starvation as there was little loss of weight in most cases. No marked sensory changes were present and it was difficult to say if the animals suffered from muscular incordination. Terminal portions of the peripheral nerves and the motor end-plates were found to be intact in the midst of necrotic muscle cells but no examination of the central nervous system was carried out. In the later stages a few of the animals show atrophic skin and falling hairs over the upper part of the thighs and sacrum. It has been noted that the disease may spontaneously arrest itself and this differentiates it from paralyses due to deficiency of vitamins B and C. If an abundant diet is given to the vitamin E deficient mothers on the day the litter is born, no paralysis results as sufficient vitamin E reaches the young through the mother's milk, but normal young weaned by the vitamin E deficient mothers do develope it. All attempts to cure the disease after its manifestation for several days failed, it being usually too late when treatment was started on the nineteenth or twentieth day of the animal's life. It could be prevented by early treatment with foods containing vitamin E and by wheat germ oil, but was unaffected by vitamins A., B, C or D. Administration of the vitamin E could be delayed until the fifteenth day after the birth of the litter. This day is apparently the critical day in the developement of the nervous system as far as the need for vitamin E in concerned. Evans considers that the sterility-curing and the paralysis-preventing factors are identical. (Evans. Burr. 1928.). These results have been confirmed. (Mason. 1933.).

Lipshutz was the first to demonstrate lesions in the central nervous system of vitamin E deficient rats. He found degeneration chiefly of the ascending and descending vestibular tracts, but also of the tecto-spinal, rubro-spinal and dorsal ascending tracts. The resulting paralysis was usually spastic although it might start as a flaccid ~~one~~ type. (Lipshutz. 1936.).

The next discovery was that old rats deprived of

vitamin E developed a flaccid paralysis of the hind limbs, with no involement of sensation, and with a normal faradic response on stimulation of the nerves to the leg muscles and of the muscles themselves. On post-mortem examination the muscles were found not to be noticably atrophic, but the sensory ganglia of the brain and spinal cord and the posterior funiculi of the spinal cord had a yellow colour which indicated pathological changes. The paralysis was apparently caused by ~~a~~ ~~both~~ both lesions in the motor and the sympathetic nervous systems. (Burr. Brown. Moseley. 1937).

The most detailed description of the lesion seen in the nervous system of vitamin E deficient animals has been given by Einarson and Ringsted. Ringsted first noted that vitamin E deficient rats slowly developed a flaccid paralysis after about twenty seven weeks, and began to drag their legs. There was also some sensory loss especially of deep-seated sensation and of the cutaneous sensation of the legs and tail. If the deficiency continues the loss of motor power and sensation increases and ataxia becomes marked. The fur falls out and the animal begins to lose weight. He noted similar changes on depriving the animals of vitamin A, but concluded that, as the food mixture was also lacking in vitamin E, the changes were ~~also~~ probably due to a long-standing and high degree of its deficiency. Vitamin E prevented the onset of the condition but did not cure it, although the preparation may not have been active. (Ringsted. 1935).

Einarson and Ringsted carried these experiments further. They fed female rats on a diet partially deficient in vitamin E, and controlling the progress of pregnancy by vaginal smears, found that in some cases a living litter was born. These young were then fed on a vitamin E free diet and were completely deficient of vitamin E in forty to fifty days. Control animals were also used and given wheat germ oil as well as the diet. The wheat germ oil was first tested for its biological effect before use, and was given before the meals. It was prepared by drying the wheat germ at 100°c for two hours and grinding it up to flour. This was mixed with freshly redistilled cold ether and stood at a low temperature in the dark for three days. Then a yellowish clear solution is filtered off by suction, and the ether evaporated by suction and by bubbling carbon dioxide through the liquid till it no longer smells of ether.

Their first observations were on twenty seven rats. Seventeen were put on the vitamin E free diet and developed paralysis and ten were also given wheat germ oil and remained normal. Seven of the first and one of the second group were killed and the rest were put on the deficient diet only to see if the neurotropic factor in the wheat germ oil was stored to any extent in the animal body. After six to nine months all had

developed the paralysis and no close relationship was noted between the amount of wheat germ oil previously given and the duration of the period before the onset of the symptoms. Since the wheat germ oil had been given however, the animals have gone through a different number of pregnancies with litters of different sizes so that this fact alone must bring about a difference in the drainage of the oil from the storage depots, affording an explanation of the different length of time of this period. No doubt individual variations in disposition to the paralysis and in consumption of the vitamin E also play a part. So the fact that the latent period before the onset of symptoms may vary from six and a half to as much as ten months is of no fundamental significance. The experiments suggest that only a small quantity of the neurotropic factor may be stored in depots and be withdrawn from these relitively slowly. It behaves like vitamin E in this respect and is no doubt identical. The paralysis never developes while the animal is still fertile and there are usually one or two resorptions first.

Einarson and Ringsted next tried to determine the exact causal factor in the production of the paralysis. Experiments show that sex plays no role in the developement of the paralyses. No signs of infection were found in the organs of the animals which might indicate a deficiency of vitamin A, and the effect of vitamin B deficiency on the nervous system differs from that discribed in these experiments. Lack of this vitamin results in spasticity, head retraction and convulsions. In some cases these changes are not cleared up unless both vitamins B and E are given which suggests vitamin B may require the co-existance of vitamin E. Lack of fats containing vitamin E results in subnormal growth and disturbance of ovulation of experimental animals, (Evans. Burr.1928.), and it has been found that linolic and linoleic acid cured disturbances of growth, trophic disturbances of the skin and gait, and effusions into the joints in similar animals. (Tange.1932). These unsaturated fatty acids also cured resorbtion and stillbirth syndromes. (Evans. Murphy.Lepkovsky. 1934.). This raised the question whether the paralysis might be due to lack of these acids as well as of vitamin E, as they were not present in the diet of the experimental animals. Howeveryforty to fifty milligrams of peanut oil and cod-liver-oil or the actual acids themselves when added to these diets did not prevent the paralysis although twelve milligrams of wheat germ oil did.

Quantities of wheat germ oil as small as six milligrams if given from the start of the experiment prevented paralysis even ~~after~~ for as long as twenty months. To guarantee protection its administration should not be started later than one and a half months after the beginning of the experiment. If it is not started for two months,

paralysis develops six to eight weeks later. It was found from these experiments that wheat germ oil could not cure the paretic condition partially or completely, but it was possible to check or limit its progression if the oil was given in the initial stages. In the later stages the paralysis progressed whether wheat germ oil is given or not and this should be kept in mind in treating similar conditions in man.
These results may be tabulated:

Period before Onset of Paresis.	Stage of Paresis at start of Treatment.	Daily Dose of Wheat Germ Oil.	Duration of Treatment.	Result of Treatment.
27 weeks.	Advanced.	125 milligrams.	4 months.	Progression of Paralysis.
24 weeks.	Early.	125 "	3 months.	No progress.
26 weeks.	Early.	125 "	2 months.	No progress.
27 weeks.	Early.	125 "	18 months.	No progress.
18 weeks.	Early.	125 "	6 months.	No progress.
17 weeks.	Early.	125 "	6 months.	Slight progress.

Period before onset of Paresis.	Addition of Wheat Germ Oil after Stated Period.	Quantity of Wheat Germ Oil.	Duration of Treatment.	Results of Treatment.
0 weeks.	One month.	50 milligrams.	4 months.	Paralysis prevented.
16 weeks.	Two months.	25 "	5 months.	Progression of paralysis.
14 weeks.	Two months.	25 "	4½ months.	Progression of paralysis.
14 weeks.	Three months.	25 "	3½ months.	Progression of paralysis.
0 weeks.	One and a half months.	25 "	5 months.	Paralysis prevented.

The development of the paralysis may be divided into four stages. First the gait becomes slow, cautious, waddling, straddling and slightly incordinated. This may be intermittent at first but finally the gait becomes permanently dragging in character. The fur on the hind quarters and legs is thin but the rats are still able to jump and are mentally lively with good appetites. Next, straddling of the hind legs is noted and ataxia developes. There is atrophy of the muscles of the hind quarters and lower extremities and the fur becomes thinner still. There may be hyperaesthesia and hypoalgesia of the skin. They are still mentally lively but move more slowly. In the third stage the hind limbs cannot be abducted and the hind quarters are

dragged along the floor. Ataxia is more pronounced and the rats cannot jump. Bald areas are seen on the trunk and hind legs. They sleep a lot but the weight remains constant. In the final stage they are unable to walk or stand, sensibility is impaired and there is marked muscular atrophy. There is the impression of marked hyperaesthesier and hyperalgesia on pinching the skin. Ulcers are seen on the skin but mentally there is still no change, although they sleep more than ever, and the animals have never been seen to die of the neurological disturbances alone even after twenty to twenty four months.

The ataxia is progressive but rarely affects the fore-legs. The loss of hair is inconsistent as it may be normal, and the disturbance of sensibility may also be absent, but the muscular atrophy is always a constant sign. Circulation in the final stages is bad, but gangrene is never seen. Incontinence of the bladder has also been observed during these stages but the bowel and uterus are apparently unaffected. If adequate wheat germ oil is given in the first two stages as soon as the animals become pregnant, living young will be born. The duration of these four stages varied in accordance with the supply of vitamin E. If there is a small but inadequate supply the duration is from six to fifteen months and the latent period before the onset of symptoms is twenty six weeks, and if the animals receive no vitamin E at all, the duration is about three months and the symptoms come on fifteen weeks after the start of the experiment.

Sometimes changes similar to those described by Lipshutz were found on post-mortem examination and these will be described first. In these adult rats the ascending and descending vestibular tracts were chiefly involved in the earlier stages, but degeneration is also seen in the rubro-spinal and the tecto-spinal tracts and in the fasiculus gracilis and cuneatus. In some case fibres in the spinal ganglia and the dorsal nerve routes were involved. The degeneration of the rubro-spinal tracts accounts for the fact that most rats show a paralysis of the spastic type. Some of them begin by showing a flaccid paralysis which changes to a spastic one. The tracts from the lateral and superior vestibular nuclei increase the tone of the lower centres and those from the medial and inferior nuclei depress this tone. So the flaccid may turn to a spastic paralysis because the former tracts are involved first. There was no evidence in these cases that the proprioceptive tracts were involved although they may be in some parts of the nervous system.

Cellular changes are seen in the anterior horn close to the central canal and in the base of the posterion horn which corresponds to Clarke's

Column in man. The cells of the medulla oblongata are also involved. It may be that these areas are more vascular than other areas and therefore use a larger supply of vitamin E. Previous experiments have never shown any cellular changes except for slight involvement of the anterior horn. In the later stages the cells of the spinal ganglia showed slight pathological changes but these may have been of a retrograde nature. In advanced cases the cells are sclerosed while fatty and vacuolar degeneration is seldom seen. However all stages from chromophil changes to complete sclerosis may be seen. The chromophil changes are irreversable and pass to chromophobe changes. They express a disturbance of intracellular processes. Chromphile changes are supposed to correspond to a state of central inhibition. Fatty and vacuolar degeneration of the cells is marked and widespread if the progress of the disease is rapid.

The changes most often found by these Authors are confined to the spinal cord, involving the proximal parts of the posterior nerve roots and the proprioceptive and possibly non-crossed tactile paths in the fasiculus cuneatus and gracilis. They correspond to the first two clinical stages in the development of the paralyses. They then describe the progression of these changes with involvement of the anterior horn cells and of the whole of the fasiculus cuneatus and gracilis corresponding to the third clinical stage. This involvement results in the degeneration of the motor fibres of the corresponding anterior roots and peripheral nerves. Examination of the muscles supplied by these showed changes typical of progressive muscular atrophy of spinal origin. The fact that normal fibres are seen in the muscle spindles and in these peripheral nerves suggests that the afferent fibres are not involved. The cells of the spinal ganglia which may show slight involement are apparently only those cells connected with temperature and pain fibres. The changes corresponding to the final clinical stage are inconsistent. The animals show a flaccid type of paralysis only and this may be explained by the partial degeneration of the cortico-spinal or pyramidal tracts which occurs at this stage and results in a flaccid paralysis if there is also a degeneration of the posterior fasiculi. The rubro-spinal and vestibule-spinal tracts are normal. Sometimes the animals show a transient rigidity in the second stage which disappears in the third. The reason for this is uncertain, but as the reflexes are absent and the red and vestibular nuclei are normal, it may be due to an involvement of the cells of the spinal cord which supply the flexor muscles before those which supply the extensor. So it is seen that the dorsal and lateral tracts are chiefly affected, and the ventral tracts to a less extent.

The tracts which are affected show a loss of myelin and a dropping out of the axons.

Cellular changes in the cortex were only seen in one animal and were situated in the deepest layers of the precentral region.

The first changes are usually seen in the lumbo-sacral region of the cord and they spread upwards through the thoracic region to the fifth cervical segment. The changes are rarely severe in the upper regions of the cord. It may be noted here that in the rats the pyramidal tracts are of minor importance compared with the rubro-spinal tracts in controlling the motor system and that the substantia gelatinosa are very large in these animals.

The parenchymatous degeneration of the cells, medullary sheaths and axons is followed by gliosis, which may be slight or marked, and by congestion of the blood vessels. If there is little gliosis the cord has a porous appearance on naked-eye examination. The gliosis is more marked if the progress of the disease is rapid.

The lesions in the lower motor neurones and the skeletal muscles may now be considered in more detail. In the first clinical stage there are no changes in the anterior horns, peripheral nerves or muscles. In the second stage the anterior horns and peripheral nerves begin to degenerate and in some cases the muscles do also. Atrophic fibres are seen scattered through the muscle with an increase in the number of their central and more rarely of their peripheral nuclei. No hypertrophic fibres are seen and there is no particular proliferation of the interstitial connective tissue. The changes are first like those of a muscular dystrophy and then of a progressive muscular atrophy of spinal origin. The anterior horn cells may be affected before the posterior funiculi. In the third clinical stage the anterior horn cells are sclerosed and the muscles markedly involved. Atrophic fibres intermingle with normal ones. Many nuclei fill out the former and the longtitudinal striations disappear although the cross striations are preserved. Longtitudinal splitting of the fibres is also seen. Finally entire muscle-bundles are atrophic and there is an increase in the connective tissue. At the end of this stage the nuclei within the fibres are almost solely confined to the periphery and the cross striation as well as the longtitudinal striations have disappeared. There is some vacuolar degeneration, but no hyaline degeneration worth mentioning. The nerves supplying the muscles show a fragmentation of their medullary sheaths

and an increase in the amount of fatty substance and in the number of histiocytes present in them.

It has been suggested that all these changes correspond to a cross between the diseases of tabes dorsalis and amystrophic lateral sclerosis. In different rats the picture may be more like one disease than another and sometimes the neuroglial reaction is so slight in the posterior funiculi that the picture is more like a case of subacute combined degeneration of the cord than either of the above diseases. Which disease the picture resembles most, may depend on the form of treatment used or on the individual constitutional properties of the animal. It has been noted that in some rats the degeneration of the pyramidal tracts is not followed by a spastic paralysis and this may correspond more closely with progressive muscular atrophy, which may be regarded as a variation of amyotrophic lateral selerosis rather than a specific abiotrophic disease.

Finally it has already been mentioned that a histiological picture like that of a primary muscular dystrophy may be present in the muscles of these animals in the early stages of the disease before the picture changes to one typical of a spinal muscular atrophy. This muscular dystrophy may be due to involvement of the fine sympathic, parasympathetic or extrapyramidal fibres to the cross striated muscles. Degeneration of the sympathetic fibres to dystrophic muscles has been noted by other Workers (Kure. 1931.). If this is so, the dystrophies described in the last section may all be cases of neurogenous and not of myogenous atrophy. Lack of vitamin E may effect sympathetic centres in the cord and this has found to be the case in some animals, anyhow, in the region of the lateral columns and intermediate zones of the spinal cord. This primary involment of the sympathetic system may be the cause of the disturbed function of the reproductive system as, if the same factors are at work, and vitamin E and the neurotropic vitamin are identical, a destruction of the sympathetic nerves to this system would undoubtedly interfere with its function. If this is so, and the first changes seen in vitamin E deficient animals are due to the involvement of the sympathetic nervous system, lesions in the central nervous system should always follow the appearance of muscular dystrophies and reproductive failure, but this is not always the case. (Einarson. Ringsted. 1938.).

When death does occur in paralysed animals from no recognisable cause it has been suggested, that, as the heart is normal, it may be due to paralysis of the respiratory muscles. These changes in the vitamin E deficient rat have been prevented by Evans on giving six milligrams of α tocopherol to the mother on the day of littering, or one milligram daily to the suckling from the tenth to the twenty fifth day of its life (Evans. 1940.).

Abnormalities have been found in the thyroids and pituitaries of these rats exhibiting neuro-muscular disorders, and these have been described before as they are the same as occur in association with reproductive dysfunction. It is suggested they might be secondary to constitutional debility. (Mason.1939b).

These pathological findings in the central nervous system of vitamin E deficient rats have been confirmed by other Authors. Mahoney describes a flaccid weakness in the hind limbs of suckling animals commencing on the eighteenth day of life, if their mothers are deficient in vitamin E. Many of them died unless they were given the vitamin, although some continued to live although paralysed. Vitamin E cured the condition. The pathology was generally similar in young and adult rats. Some of the nerve cells in the anterior and posterior horns and in fact all the grey matter of the cord, as well as in the medulla, pons, cerebellum and brain, showed signs of degeneration as did the fibres arising from these cells. The cytoplasm was hyperchromatic with loss of the Nissl pattern. The resulting overstaining obliberated the nuclei and nucleoli. The cell processes were heavily stained for long distances and the cell bodies were generally thinner and longer than normal, with sharply outlined margins. There were no phagocytes around the cells, but some were in the process of disintegration and stained faintly. They contained vacuoles and had lost their Nissl pattern, and in the very late stages some were phagocytised. The changes did not resemble any typical a retrograde reaction of Nissl or chromic cell changes.

The Sciatic nerve was examined and found to show a loss of its myelin sheath and alteration in the axis cylinder, with involvement of both its anterior and posterior routes in the corda equina. The long conducting tracts in the cerebrum, cerebellum and spinal cord were similarily affected. The antero-lateral tracts, and in particular the spino-cerebellar and vestibulo-spinal tracts, are chiefly involved in the cord; and the corona radiata, and the median forebrain bundle in the brain. The changes were present in the upper as well as the lower segments of the cord and there was a glial reaction in all areas. The cell changes occur first, then the nerve paths degenerate and finally the muscles supplied by these nerves atrophy. Vitamin E prevents the onset of these phenomena. (Mahoney.1941).

Other Experimentors have found that crude preparations of vitamin E, or α tocopheral, both prevented the development of ~~the~~ paralysis in vitamin E deficient animals (Morelle.1931.), (Demole.1940.), (Sheldon. Butt. Waltman. 1940.).

The Cerebrum.

It has already been noted that in some cases the cells and tracts of the brain show signs of degeneration under conditions of vitamin E deficiency in the rat. (Einarson. Ringsted.1938.), (Mahoney.1941.).

When chicks are deprived of vitamin E more extensive changes are observed. Nutritional encephalomalacia has often been observed in chicks before but it was not certain if this was due to lack of vitamin E or some other factor in the diet. (Dam. Glavind. Bernth. Hagens. 1938.). Adamstone noted that if hens were put on a diet deficient in vitamin E their chicks showed a condition of imbalance and impaired musculature three to four weeks after hatching, if fed on the same diet. On autopsy the brain was found to show signs of haemorrhage and disorganisation in a certain number of the cases. Some of the chicks made a slow spontaneous recovery. On microscopical examination haemorrhages, degeneration of the cells and breakdown of the fibres were seen. The changes occured mainly in the cerebellum but also in the cerebrum and brain stem. They were quite different to those seen in states of vitamin B and D deficiency but somewhat similar to those of vitamin A deficiency, although no really gross changes occur in the brain in the later case. From experiments it was found that the changes were not due to the ether or ferric chloride used in the treatment of the diet. Vitamin E provides a certain protection against these changes but does not entirely prevent them. It is suggested that wheat germ oil, or α tocopherol, may be only one of the factors needed for this. If the diet is not heated to evaporate the ether, the changes are not produced, although the vitamin E may be destroyed by other methods, so it is possible that the heating may destroy some unidentified factor. However it appears essential that vitamin E must be destroyed as well, because just soaking the food in ether and not treating it with ferric chloride before heating, results in no demonstratable changes. The erratic nature of the disease and the difficulty in preventing it by the addition of vitamin E to the ~~diet~~ food certainly suggests that there may also be a lack of some heat labile substance in these animal's diets or a failure or inability in them to use this substance, even if it is present, under conditions of vitamin E deficiency. (Adamstone. 1941a.). The possibility of there being two anti-encephalomalacic factors, one fat soluble and one water soluble, has ~~also~~ been suggested by other Authors. The fat soluble factor is almost certainly vitamin E and it is possible that the vitamin might be standardised by this activity. (Dam. Glavind. Bernth. Hagens. 1938.).

The encephalomalacia in vitamin E deficient chicks has been prevented by α tocopherol alone by

some Experimentors so this suggests that the second factor may only be necessary under certain conditions. The necrosis of the brain seen in these experimental animals may possibly be due to prolonged vaso-constriction due to involvement of the autonomic nervous system. (Pappenhiemer. Geottsch. Junghenn. 1939.). (Evans, 1940.).

The Cholesteral content of the brain of these vitamin E deficient chicks was found to be less than normal. This is first noted during the third week of life and after that the cholesteral content falls rapidly. So this week is apparently a critical period for the maintenance of normal cholesteral metabolism in the brain of chicks. There should normally be an increase in the cholesteral content of the brains of developing chicks during the second week of life but as is seen, this does not take place under conditions of vitamin E deficiency. An increase in the cholesteral content of the brain of vitamin E deficient rabbits has been noted. (Mackenzie. Wilder. Spencer. Eppstein. 1938.). It has already been stated that in the cases of diseases of the reproductive system and of muscular dystrophy resulting from vitamin E deficiency the metabolism of cholesteral or closely allied substances is disturbed, and the facts noted above are a further link in this relationship. No explanation of the rôle that vitamin E plays in the metabolism of these substances has been offered. (Adamstone. 1941,e.).

To complete this section the effect of vitamin E deficiency on the ears of experimental animals may be described. Under these conditions the otic capsule has been found to show irregular localised thickening, and the fibres of the muscles of the middle ear to be atrophic. The animals were found to be very subject to middle ear infections so the muscular changes may be due to this and not necessarily to deficiencies in the diet. (Covell, 1942.). Vitamin E is also apparently concerned in some way with the normal function of the semicircular canals as animals deprived of it show signs and symptoms indicating that part of these canals are affected by the lack. The rotatory movements and abnormal positions of the head indicate that the transverse system is mainly involved. (Lipshutz. 1938.).

Growth and Weight.

It was suggested by Evans that vitamin E did not exert the favourable effects upon growth and the general state of health of his experimental animals indirectly through its established value to the sex glands, but in some possibly more direct way. He found that wheat germ oil, added to a diet deficient in vitamin E, improved the growth and vigour of the animals if given within one to two days of the first appearance of any symptoms, even if it was not present in sufficient quantities to cure their sterility. This improvement

was only seen after the eighth month of life so presumably the vitamin is not necessary for the growth of these animals before that time. Growth is not interfered with by removal of the sex glands so vitamin E cannot exert its influence through these. (Evans.1928b.). Blumberg also noticed a retardation of growth in young rats, deprived of vitamin E, at the twelth to fourteenth week of life with complete cessation at the eighteenth to twenty second week. Serious malnutrition and muscular weakness resulted if the deficiency continued till the thirtieth to fortieth weeks. These signs were prevented by vitamin E. (Blumberg.1935.). These findings have been confirmed by other workers. (Emerson. Evans. 1937.), (Olcott. Mattill. 1937.).

Other Authors have noted that the final body weight, total gain in weight and the amount of body fat is less in vitamin E deficient animals. These results may be tabulated:

Number of Rats.	Dose (Weekly).	Period (Days)	Mean Initial Weight. Grams.	Mean Final Weight. Grams.	Mean Weight Increase.	Mean Daily Weight Increa
3 Female.	50 mg. W.G.O.	261.	138.	244.	106.	0.41.
3 Female.	Nil.	261.	148.	206.	58.	0.21.
3 Female.	50 mg. W.G.O.	234.	37.	189.	152.	0.65.
3 Female.	Nil.	234.	40.	162.	132.	0.52.
3 Female.	50 mg. W.G.O.	147.	127.	233.	106.	0.72.
3 Female.	Nil.	147.	122.	161.	39.	0.27.
2 Male, 2 Female.	3 mg. α tocopheral.	105.	149.	278.	129.	1.23.
2 Male, 2 Female.	1 mg. α tocopheral.	105.	142.	253.	111.	1.06.
2 Male, 2 Female.	0.3 mg. α tocopheral.	105.	146.	264.	118.	1.12.
2 Male, 2 Female.	0.1 mg. α tocopheral.	105.	130.	229.	99.	0.94.
4 Male, 4 Female.	Nil.	105.	129.	226.	97.	0.92.

The same Authors noted that, on treatment of rats paralysed due to deficiency of vitamin E, the paralysis was often unaffected but the weight was increased and the growth became more rapid. (Moore. Martin. Rajagopal. 1939.).

The stimulation of growth and weight by vitamin E has been confirmed by various Experimentors. (Dam. Glavind. Bernth. Hagins.1938.), (Mackenzie. McCollum. 1941.). The failure of growth and the maintenance of body weight in vitamin E deficient animals may be due to the alterations found in the thyroid and pituitary glands of these animals. (Underhill.1939.).

On the other hand some Experimentors state that the growth-promoting factor of the fraction of wheat germ oil rich in vitamin E, which undoubtedly seems to stimulate growth of young vitamin E deficient rats, is

not the same as the anti-sterility one. They could destroy the growth-promoting, but not the anti-sterility factor, by heat. (Martin. 1937.). Evans and Burr in their original experiments on vitamin E deficient rats found no difference between the growth of treated or of untreated animals and concluded that the vitamin was not necessary for growth; However it must remain an open question till further experiments are carried out. (Evans. Burr. 1928.).

(b). In Human Beings.

There is no doubt that lack of vitamin E results in lesions of the neuro-muscular system of experimental animals, but there is no proof that human beings suffer from diseases due to a similar deficiency. However, the resemblance between the experimental disorders and spontaneous nervous and muscular diseases in man indicated a trial of the vitamin in their treatment; although it must be remembered that even in animals the nervous lesions were rarely cured by the vitamin, so improvement would only be expected in early cases.

If these diseases do result from lack of vitamin E it must be proved that the supply of the vitamin is deficient in the case of some people's diet. This problem has already been considered. It may be that certain people, owing to some idiosyncrasy, or to the associated presence of an infective or toxic agent, require greater quantities of the vitamin than normal for the maintainance of their neuro-muscular systems, or they may actually consume a diet deficient in the foods which are rich in it. (Bichnall. 1940.). However it has been noted that there are usually no signs of dietary deficiency in cases of tabes dorsalis, muscular dystrophy, amyotrophic lateral sclerosis or muscular and nervous atrophy in man, which are diseases that have been stated to be possibly due to lack of the vitamin. (Denker. Shememan. 1941.), (Taylor. 1940.). Until it is proved that there is a minimum quantity of the vitamin essential to the human body this question cannot be settled.

The difficulty in estimating the amount of vitamin E present in the blood in health and disease, and the scanty investigations carried out on normal subjects, makes the problem of whether lack of vitamin E results in any untoward effects in the human being, one of pure hypothesis.

The individual diseases which have at one time or another been attributed to lack of vitamin E will now be considered.

Muscular Dystrophies.

Primary muscular dystrophies in man are divided into various groups such as Charcot - Marie - Tooth peroneal muscular atrophy, pseudohypertrophic muscular dystrophy and Erb-Landouzy scapulo-humeral dystrophy, but the division is an anatomical ~~one~~ rather than a pathological one. However the juvenile muscular dystrophies compare most closely to the purely muscular dystrophies, without nervous changes, which are seen in young rabbits and guinea-pigs deprived of vitamin E. As a result of this it has been claimed that these conditions in man are a deficiency disease and therfore curable. (Bicknall.1940.). True pseudohypertrophic muscular dystrophy is never seen in experimental animals but the theoretical background for the use of vitamin E in the treatment of these diseases is sounder than in any other nervous disease and amply justifies its trial. For the sake of comparison a brief description will be given of the typical changes found in dystrophic human musculature. The muscle fibres are of different shapes and sizes, and in longitudinal section they are seen to be fragmented into two or three segments, each of which may be surrounded by a number of sarcolemma nuclei. This increase in the number of the nuclei of the muscle fibre is characteristic and it is the central nuclei and not the marginal hypolemmal nuclei which are mainly involved. Hyaline degeneration and vacuolation of the larger hypertrophic fibres is common, and they may become fibrous near their tendinous attachment. As the degeneration progresses the muscle tissue becomes replaced by connective tissue and fat, until only a few scattered fibres, some of which are larger and more swollen than normal, are seen. There is also some perivascular round-cell infiltration. (Aring, Cobb, 1935.). It has already been mentioned that ~~the~~ muscular dystrophies may be caused by degeneration of the autonomic nervous system but no support for this has been found in the cases of muscular dystrophies in man.

From experimental findings it is obvious that the main rôle of vitamin E in the treatment of nervous diseases must be a prophylactic one, as once the condition has developed the vitamin may not even arrest it, much less cure it. For this reason it has been suggested that vitamin E be given to mothers who have previously given birth to children suffering from muscular dystrophies. In rats the neuro-muscular degeneration does not appear till about the twenty first day of life and, if the previous history of the mother was not known, the origin of the degeneration could not be ascertained although histiological studies would have revealed muscular changes some time before the onset of the symptoms. Myopathies also appear after a lapse of some years in children and, if a careful investigation could be made, it might be found that the diet of the

mother was deficient in vitamin E. As sensory organs such as the olfactory and auditory nerves, and the intelligence, may suffer in animals deprived of vitamin E it is possible that this congenital Avitaminosis might also be an etiological factor in certain disturbances of the higher centres in children. Premature infants would be especially liable to develope these diseases at a later date because prematurity itself may possibly be caused by lack of vitamin E. The slow gain in weight sometimes seen in these children may also be due to a similar cause. Familial dystrophies would be unlikely to improve, as some genetic factor must play a large part in the etiology of these cases, although a vitamin deficiency might have an added detrimental effect.

The theories that the nervous system of experimental animals are unable to function normally unless vitamins E and B are present at the same time, and that vitamin B may potentiate the action of vitamin E, have already been mentioned. These theories have also been applied to the treatment of Human Beings. (Stone. 1940.). Improvement in such conditions as muscular dystrophies, epilepsy, myesthenia gravis, and post-encephalitic parkinsonism have been noted after treatment with vitamin B6; but the results of the treatment of nervous diseases on giving water soluble and fat soluble vitamins together, are no better than when vitamin E is given alone. (Spies. Hightower. Hubbard. 1940.), (Antopol. Shotland. 1940.). The suggestion that muscular dystrophy is an abiotrophic lesion, is untenable owing to the wide distribution of the lesions.

Many Authors have reported favourably on the treatment of muscular dystrophies and these reports will be given later. However other Investigators think that there are no grounds for using the vitamin in these conditions and have never seen cases responding favourably to the treatment. (Hume. Henderson-Smith. 1938.), (Shute. 1939.), (Wechsler. 1940.). There is a big psycological factor in these diseases which makes it difficult to asses treatment; but Wortis suggests that they are probably due to a multiple deficiency, or possibly to a synergic action of the vitamin, a question that has hardly been touched upon. The vitamin is also a drug, just as nicotinic acid is, owing to its vasodilator action, so any improvement noted may be due to a side action of this nature which may stimulate muscular metabolism, or to a psycological effect, and not to the improvement of a deficiency. As in experimental animals the vitamin only cures the nervous phenomena if given at an early stage, it is obvious that treatment can only be expected to be successful in cases diagnosed soon after their onset. (Wortis. Jolliffe. 1941.). Another difficulty

in assessing the value of vitamin E in the treatment of these diseases is the wide variations in the course of their developement. They may suddenly develope and remain stationary for years at a time, and possibly show some spontaneous improvement, although this may only be subjective. Before definite conclusions could be drawn the treatment would have to be continued for years and careful observations taken during this time. (Doyle. Merritt. 1941.). Several cases of muscular dystrophy and sterility occuring together in the female patient have been noted and when they were given a richer diet they became pregnant, although there was little increase in the muscular strength. (Mahoney. 1941.).

Finally it may be noted that Taylor found that muscular dystrophies, especially of the pseudohypertrophic type, were common among the people of Lahore, whose staple article of diet consists of 'Chapeth' made from whole wheat which presumably contains adequate amounts of vitamin E. He also occasionally met with cases of tabes dorsalis and amyotrophic lateral sclerosis among these people, both of which diseases have been considered possibly to be due to lack of the vitamin, but the incidence of these was not high enough to draw any conclusions from. However the high incidence of the muscular dystrophies led him to believe that it was unlikely that vitamin E played any part in their cause. (Taylor.1940.).

Creatine and Creatinine Matabolism.

It has already been noted that there is an increase in the urinary creatine, in context of animals when they develope a muscular dystrophy, and that this falls if they are improving under treatment with vitamin E. So it was thought that the response to vitamin E of humans suffering from muscular dystrophies might be gauged by estimating the quantity of this substance in the urine. The results have been disappointing as they seem to show that vitamin E, and B6, are valueless in the treatment of these diseases, but details will be given when the cases are discussed. (Bang. 1941.).

Creatine is normally present in the urine of children in moderate amounts, and to a less extent and irregularly in the urine of women, but it is not present or only in very small quantities in the urine of male adults. The excess in the urine in cases of muscular diseases has been stated to depend directly on the amount of improperly functioning muscle in the body. (Milhorat. 1937.). This can only be the case if the subject is on a creatine-free diet, as the capacity to store injested creatine depends on the mass of efficient muscle present, the diseased muscle being unable to store it.

Valuable information should also be obtained by an estimation of the urinary creatinine but this has not been the case in practice. The daily output of creatinine in the urine is an index of the amount of properily functioning muscle in the body. It should be constant from day to day in any one individual but varies with age and sex, depending on the mass of muscle present. The quantity present is greater in adults than children and in men than women.

The creatine and creatinine output in the urine is probably only an approximate index of the healthy and diseased muscle present in the body, but a significant change in the clinical state of the patient should be reflected in this output. When the patient improves the creatinine output should rise and the creatine fall and visa versa. If the disease progresses the output of both falls concurrantly.

Fitzgerald and McArdle found that the creatinine index of females was fourteen to twenty two, and of males twenty to twenty six. It fell in both sexes when suffering from muscular dystrophies. Patients suffering from both motor neurone disease and muscular dystrophies showed fluctuations in their creatine - creatinine output which were greater than normal. These may have been spontaneous and the result of the disease process, or have been due to the vitamin E therapy, or due to the incomplete collection of the urine. The absence of a definite trend in the fluctuations made vitamin E therapy as the cause unlikely. The quantity of the urine may have been incomplete, but the creatine, as a percentage of normal, should have been unaffected in that case. So they were probably due to the disease.

They found no improvement in the creatine - creatinine metabolism in cases of motor neurone disease, or of muscular dystrophy, on treatment with vitamin E or with vitamins E and B6. Minor fluctuations of no significance were noted in mild cases, and in one case there was a rise in both the creatine and creatinine output, but this was probably due to inefficient collection of the urine. (Fitzgerald. McArdle. 1941.). Abnormal fluctuations in the creatine - creatinine output in cases of muscular dystrophies in humans have been noted by other Authors, (Ejserson. Thomson. 1937.), and denied by some. (Adams. Power. Boothley. 1935.).

It is suggested by certain Investigators that the fact that vitamin E influences the creatine - creatinine excretion in experimental animals suffering from muscular dystrophies, while it has no effect on the excretion in Human Beings suffering from the same type of disease, indicates a fundamental difference between the pathogenesis of the two conditions. (Fleischmann. 1941.), (Harris. 1941.).

Motor Neurone Disease.

Amyotrophic lateral sclerosis, progressive muscular atrophy and bulbar palsy are all different types of this same disease. It has also been claimed that this is a deficiency disease and therefore curable. (Bicknall. 1940.). The theoretical background for the use of vitamin E. in the treatment of this condition is not so good as in the case of muscular dystrophies. The findings of Einarson and Ringsted in vitamin E. deficient rats have already been described, and the fact that they suggested that these resembled a cross between amyotrophic lateral sclerosis and tabes dorsalis in man, noted. However the similarity is not very close either clinically or pathologically.

In rats the first changes are often sensory, then the gait becomes atoxic and finally weakness and paralysis supervenes, but in most cases only in the hind limbs, although gross static tremors and incordination of the fore-limbs have been noted by some Authors. (Mackenzie. Mackenzie. McCollum. 1940.) . The pathological picture is one of initial degeneration of the posterior columns and later of the anterior horn cells and, only inconsistantly and in the final stages, of the pyramidal tracts. The rubro-spinal tracts are rarely involved.

In amyotrophic lateral sclerosis the clinical picture is essentially one of motor involvement, the paralysis being either spastic flaccid or mixed. The pathological picture shows a degeneration of the upper motor neurone including the Betz cells, and of the lower motor neurone, with a consequent atrophy of the muscle supplied. There is a gradual onset of weakness, pain and stiffness from either upper or lower motor neurone degeneration, and fibrillary twitchings are a diagnostic sign; the weakness is most common in the upper limbs. The signs of atrophy in the white matter of the cord are diffuse except in the posterior columns, which are hardly ever involved, and chiefly affect the upper motor neurones and intersegmental fibres. The supporting tissues show signs of a proliferative reaction, and the tracts themselves a loss of myelin and a dropping out of the axon cylinders. The degeneration of the lower motor neurone cells may take place throughout the ventral horns and homologous nuclei in the brain, especially in the cervical and lumbo - sacral segments. Most of these cells are small, angular and pyknotic, and there is a diminution in the total number present. Pathological lesions are sometimes seen in Clarke's Column. In the muscles the degeneration is irregular. The fibres are shrunken with an increased number of central and marginal nuclei and lie side by side with normal ones.

Cross stiations can usually be made out in the atrophic fibres and they sometimes show longtitudinal splitting. There may be signs of hyaline and vacuolar degeneration, and a few of the fibres may be hypertrophic. Fibrosis is marked but fatty infiltration is not. The muscles of the hands are those most often involved.

The disease undergoes exacerbations and remissions and there is usually some emotional instability. The most outstanding difference between the conditions in man and in animals is the involvement of the Betz cells of the cortex, with subsequent gliosis, in the former. This was practically never seen in experimental animals. (Wohlfohrt. 1932). The possible explanation for this is the relatiue unimportance of the pyramidal system in the animals.

Those Authors who report favourably on the treatment of motor neurone disease with vitamin E. admit that treatment must be started early if recovery is to take place and Wechsler states that if the case is going to recover it recovers quickly, the most recently developed signs being the most likely to receed. Muscular tremors are often the first sign to disappear. The absence of the vitamin acts in a specific way on a special tissue, if it has any action at all in man, so one would expect the vitamin to also act specifically in a curatiue way and result in a quick recovery. He also states that there may be a subclinical lack for a long time and then, owing to some toxic or infectiue cause which increases the demands of the nervous system for vitamin E., the appearance of symptoms may be suddenly precipitated. He noted that the incidence of amyotrophic lateral sclerosis is greater in males than females but did not know if the males suffering from this disease became sterile, although there was no loss of libido. Vitamin E. deficiency in animals is easier to treat in females than males, possibly due to an endocrine cause, but Wechsler did not know if this was the same in humans. However in his cases five out of the six cases which showed marked improvement on treatment with vitamin E. were females of premenopausal age and the other was a male. There was some improvement in four males and one female of premenstrual age and the disease was arrested in one male and two females of post-menopausal age. Two males and two females of post-menopausal age were worse under treatment and two males died.

Wechsler thought that Amyotrophic lateral sclerosis might arise from several factors such as allergy, vitamin deficiencies, a previous attack of encephalitis or vascular disease, or ~~to~~ a low grade luetic infection.

In old patients certain bulbar syndromes are indestinguishable from the bular palsy type of this disease and so one should not expect this type of case to be often cured with vitamin E. The fact that a nervous deneration of this sort might be the end result of various factors, a particular one attacking a particular part of the nervous system, suggests that the results of treatment with vitamin E. are bound to be erratic, but gives justifiable grounds for its use in a variety of conditions. He concludes that vitamin E. can be expected to cure only one of the three or four possible types of amyotrophic lateral sclerosis which he considers to exist, and that the type due to vitamin E. deficiency probably accounts for about a quarter to a third of the cases in any one series. It may be mentioned here that treatment of this condition with vitamins A.B.C. and D. have always given negative results, although it is suggested that vitamin B. may aid vitamin E. in its treatment. (Wechsler.1940.b., 1941.a.).

Patients who suffer from this disease rarely show that they have been taking a deficient diet and some of those Authors who report favourable on its treatment with vitamin E. account for this by suggesting that the nervous systems of these patients has a higher demand than normal for the vitamin, due to a heriditary disposition or toxic influence. They also suggest that the part of the nervous system to suffer from the deficiency depends on the age of the patient and whether any nervous tracts are already weakened by other causes. They consider that amyotrophie lateral selerosis and muscular dystrophies are the same deficiency occuring at different ages. (Bicknell.1940.).

The same difficulties, as in cases of muscular dystrophy, arise in trying to assess the results of treatment of this disease with any therapentic agent. The course of the disease may remain stationary for long periods at a time, and may subjectively improve. Even if it is proved that vitamin E. deficiency does not exist in man, it does not rule the vitamin out as a therapentic agent in these conditions as it may have some side action which is of curative value.

Finally an attempt to estimate the tocopherol level in the serum of normal people, and patients suffering from amyotrophic lateral sclerosis, may be mentioned. The estimations were made by the Emmerie and Engel method. Twelve normal people showed a tocopherol level in their serum of 0·59 to 1·62 milligrams per a hundred millilitres of serum with an average of 0·96 milligrams. Thirteen patients suffering from the

disease, four of whom had had no treatment with vitamin E, showed values of 0·52 to 1·0 milligrams per a hundred millilitres of serum, which was within normal limits. However when ephynal and wheat germ oil were given orally and intramuscularily the values went up to 1·13 to 2·26 milligrams, showing that there is an upper threshold of about 2·0 to 2·5 milligrams per a hundred millilitres of serum as they never went higher than this. On stopping the treatment the serum content of the tocopherol returned to normal. There was only an improvement clinically in some of the cases treated so this suggest that the disease cannot just be due to a lack of vitamin E., but there may be a barrier to the passage of tocopherol from the blood stream to the nervous and muscular cells or some factor may stop the normal utilisation of the vitamin. (Wechsler.1941b.).

Tabes Dorsalis.

It has already been stated that in certain cases the pathological changes in the nervous system of experimental vitamin E. deficient animals resembles those seen in Tabes Dorsalis. Also the suggestion that in certain cases the nervous system demands a greater supply of vitamin E. than normal has been discussed. So it is possible that in Tabes Dorsalis a degeneration due to vitamin E. deficiency is taking place in nerve tracts weakened by syphilis, and this might explain why some syphilitic cases advance to this disease while others do not, but develope a chonic meningitis or a generalised paralysis. These facts make it justifiable to try vitamin E. in its treatment although the results have been disappointing. (Bicknell.1940.).

Subacute Combined Degeneration.

Shute reports a case of myelopathy following pernicious anaemia in which wheat germ oil had a favourable effect, and he also suggests that all mild forms of myelopathy, such as acroparaesthesias, may yield to treatment with vitamin E., although there is little theoretical evidence for this suggestion except that vitamin E. may be of value to any nervous cell which has been damaged by disease. (Shute.1939.).

Poliomyelitis and Polyneuritis.

Vitamin E. may be of value in the treatment of these conditions for the same reasons as given in the last section. It has been tried but with little success. (Stone.1940.).

Cerebral Palsies.

Infantile cerebral Palsies might be due to a vitamin E. deficiency of the mother before the birth of the child. In some of these cases no definite cause,

such as trauma or haemorrhage, can be found and these might be due this deficiency, but it cannot be easily proved as the therapy would have to be given to the mother during the latter months of pregnancy. However it might possibly be done, by taking a large number of pregnant women who were receiving vitamin E., and a similar series who were not, and calculating the incidence of infantile cerebral palsies in the children of each group. The vitamin might also be given to the new-born child in the hope of preventing further disability and of possibly correcting the condition itself. The results of such a trial has not been recorded. (Mahoney, 1941).

Fibrositis.

Fibrositis following the degeneration of their muscles due to vitamin E. deficiency has been noted in experimental animals, so it was suggested that vitamin E. be tried in the treatment of certain types of fibrositis in man. ~~This~~ fibrositis may be a primary inflamation of the connective tissue, or be secondary to such conditions as atrophic arthritis or gout, and as might be expected the vitamin has been found to be of some value in the treatment of the first type. Primary fibrositis may be a metabolic rather than an infective disturbance. (Steinberg, 1941).

Calcification of Tendons.

In a similar manner calcification has been seen to follow degeneration of the muscles of vitamin E. deficient animals, and it was thought that possibly vitamin E. played a part in the etiology of the calcification of tendons in man, especially of the tendioscapular tissue. Sometimes there is no history of trauma to account for these cases, or if there is it may precipitate calcification in tissues which have degenerated and necrosed owing to lack of vitamin E., but this is pure hypothesis.

It has already been stated that in animals deprived of vitamin E. the muscles may show pathological lesions before any symptoms arise, and the same may apply in humans as calcification of tendons can often be demonstrated although it is not affecting the patient in any way. The degeneration of tendons, such as the supraspinatus and gluteus medius tendons, which occurs in old age, is not followed by calcification, which seems to indicate a different etiology in these cases.

In view of these suggestions α tocopherol was used in the treatment of this disease and found to have no special effect on tissue which

had already undergone calcification. However it seemed to possibly influence non-calcified necrotic tissue and to prevent its calcification; anyhow in the tissues of the supra - spinatous tendon. (Sutro. Cohen. 1941.).

Malnutrition and Lordosis.

Striking improvement has been recorded in the treatment of chronic ill-nourished people with α tocopherol, but it may easily have been some other factor which brought this about. (Spies. Viltner. 1940.). Cases of Lordosis have also been successfully treated with vitamin E., and if this is a genuine case of cure due to the vitamin, it may be that the muscles of the back are increased in strength due to this treatment, if they were suffering from a mild degree of degeneration. (Donovan. 1940.).

Miscellaneous Diseases.

Other neuro-muscular diseases have been treated with vitamin E, such as amyotonia congenita, myotonia congenita, myotonia atrophica, myesthenia gravis, paralysis agitans, post - encephalitic parkinsonism, chemical intoxications of the nervous system, thyrotoxic myotrophies, and pink disease. The theoretical evidence for this treatment is non-existent and no favourable results have been recorded.

(c) Theories on the Mode of Production of the Effects of Vitamin E Deficiency in Man and in Animals.

It will be seen from the experimental evidence recorded above that there seems to be no doubt that lack of vitamin E, even in its purest form of tocopherol, causes lesions in the neuro-muscular systems of certain experimental animals, and that tocopherol is the myoneurotropic factor, (Evans. 1940.); although some Investigators have stated it is not, being merely present along with this factor in wheat germ oil. (Bicknell. 1940.). Mention may again be made of the suggestion that two different parts of the tocopherol molecule are concerned with these two different systems, or that α tocopherol is concerned with the neuro-muscular system and β tocopherol is concerned with the reproductive system. It has also been stated that whether diseases of human beings showing a somewhat similar pathology to these conditions in animals are due to a similar deficiency, is open to grave doubts. However the theories that have been suggested to explain the mode of production of the lesions in the neuro-muscular systems of both man and animals, that may

be due to vitamin E. deficiency, will be discussed. Much less study has been given to this question than to the similar question as it applies to the reproductive system.

The Cytological Theories.

The fact that vitamin E. might be needed for normal nuclear function has already been discussed but it is a theory that is particularly applicable to the case of the nervous lesions produced by vitamin E. deficiency. If it is needed for all cells, and not just for certain types, the degeneration of the nervous cells may be regarded as an acute manifestation of a deficiency disease in tissues whose vitamin E. requirements were high at the time when they were affected, due to some ~~other~~ cause such as an associated toxaemia or infection. Rapidly dividing cells would no doubt need more of the vitamin than others, which would account for the frequency of lesions in the muscles of the young; although this may also be due to involvement of the cells of the autonomic system. It is also suggested that, if severe lack of the vitamin results in neuro-muscular lesions, milder grades of deficiency may only interfere with the growth and vigour of the animal due to thegenerally depressing effect of its lack on the cells. (Stone. Manchester. 1940.). This supposed depressing effect of vitamin E. deficiency makes it possible that the vitamin may be of value in the treatment of all degenerative conditions of the muscles and the nervous system, such as disseminated sclerosis. Certainly a sick cell should have an abundance of all that is necessary for life.

It has been seen that vitamin E. may also be needed in the metabolism of the cell, although the end result would be the same whether the nucleus or the metabolism was affected. The suggestion is that the vitamin developes a quinone-hydroquinone equilibrium as an essential feature of its metabolism and so takes part in the oxidation-reduction processes of the cell. This might be especially applicable to the metabolism of vitamin C. and it has been shown that the oxidation of ascorbic acid by peroxideperoxidase is accelerated by the quinone group. (Tauber. 1936.). Further experiments will be needed before it is settled exactly what part the vitamin plays in muscle metabolism, and the prevention of its degeneration, but the fact that it apparently controls the creatine excretion in experimental animals suggests it does play some

part. Support is also given to this hypothesis by the fact that it has been found in vitamin E deficient experimental animals that the number of muscle fibres damaged is inversely proportional to the vitamin E intake, although the absolute number varies in different animals. (Mackenzie. Levine. McCollum. 1940.). Finally the possibility may be mentioned that the vitamins act synergistically to one another in cellular metabolism and so account for the apparent interrelationship between such vitamins as B and E, that has been suggested by some Authors.

Pituitary Dysfunction.

Abnormalities in the pituitaries and thyroids of vitamin E deficient animals suffering from neuro-muscular lesions has been described, but it is thought that these are probably not primary changes but due to constitutional debility. (Mason.1939b.). It is difficult to see how the pituitary dysfunction could have such a profound effect on the nervous system and no explanation has been offered by experimental workers. As a result of his experiments Barrie thinks that the paralysis is connected in some unknown way with a failure in the function of the anterior pituitary and states that in its early stages this cannot be due to a severe lesion as the condition can be cured by large doses of vitamin E. (Barrie.1938c.). The relationship between nervous lesions due to vitamin E deficiency and the endocrine glands gains some support from the more than accidental occurance of muscular dystrophies with Fröhlich's syndrome. This also brings up the question whether vitamin E indirectly controls fat metabolism through its function of preserving the cells of the pituitary in a normal state. (Stone.1940.). If pituitary dysfunction does not cause the neuro-muscular lesions it is unlikely to cause the reproductive ones either, as all the effects of vitamin E deficiency should be able to be explained by one theory.

The Sex Hormones

It has been noted that the neuro-muscular lesions are easier to treat in the female, but whether this has any connection with the fact that the ovaries are not affected by vitamin E deficiency, while the testes are, ~~by vitamin E deficiency~~ is not known. It may also be mentioned that the incidence of muscular dystrophies and amyotrophic lateral sclerosis is greater among males than females.

Shute's theory of the presence of an anti-proteolytic factor in the blood in states of vitamin E deficiency has already been fully discussed. In the case of the reproductive system it is the only theory which really explains the cause of the

effects of vitamin E deficiency without recourse to some unknown factor, so if the neuro-muscular lesions could also be explained on this basis it would be of some value.

It has been noted than an insufficiency of proteolytic enzymes in the blood results in cases of muscular dystrophy, (Meddalesi.1936.), so if this is the case, a similar state of affairs would arise owing to the presences of Shute's antiproteolytic factor. Vitamin E inhibits the presence of this factor so would be expected to cure both nervous and reproductive diseases. Possibly Shute's factor interferes with the normal metabolism of the proteins and prevents their transformation into normal muscle tissue. (Stone.1940.).

In support of a relationship between, the effects of vitamin E deficiency on the nervous system, and the gonads, it has been demonstrated in rabbits that the testes and ovary undergone progressive degeneration during the developement of muscular dystrophies, but this may be a by-product of a general metabolic disturbance as testosterone and anterior pituitary substance have no effect on the dystrophy. (Morgulis. Richards.1941.). The fact that sterility occurs in cases of dystrophia myotonica may also be mentioned. Cases of sexual maldevelopement have been successfully treated with vitamin E, but no results of the treatment of diseases involving both the nervous and the reproductive systems have been given.

A Nutritional Barrier to Virus Infections.

By the intramuscular injection of the vesicular stomatitis virus into mice it was found that nutrition played a part in the development of a constitutional barrier to the involvement of the nervous system by certain viruses. It is not known for certain what factor, owing to its deficiency, causes a lowering of the efficiency of this barrier but it is thought to be most likely vitamin Bi, riboflavin or vitamin E. If it was vitamin E it might explain the possible action of the vitamin in the etiology of such diseases as anterior poliomyelitis, encephalitis and disseminated sclerosis, and give some basis for treating these conditions with this vitamin. (Sabin. Duffy. 1940.).

(d). The Application of vitamin E to Clinical Medicine.

1. Muscular Dystrophies.

The reports of Authors, who recorded

favourable results on treating muscular dystrophies with vitamin E, will be given first. Bicknell treated eighteen of these cases, most of whom were children, with half an ounce of fresh whole wheat germ twice a day. Massage was also given in nearly every case. They were treated from twelve to eighteen months and twelve showed definite improvement and six did not. The twelve successful cases should have got worse or their condition should have remained stationary, but instead they very slowly recovered and in one case the recovery has lasted for eighteen months. He states that there is no reason why in early cases the recovery should not be complete if the treatment is prolonged; and even cases who were bedridden showed some signs of improvement although contractions might have to be dealt with surgically. One of the cases relapsed when treatment was stopped. There were no ill effects or sexual stimulation during the treatment. The six failures were all advanced cases and except for one case, who was mentally deficient, and received four months treatment, none of them received more than six weeks treatment. One of these cases died. These results lead Bicknall to consider that muscular dystrophies are a deficiency diseases and therefore curable. (Bicknell. 1940.).

Stone treated thirteen cases of muscular dystrophy most of which were ambulatory. Only five of these cases were treated long enough for definite conclusions to be drawn from the results of treatment. To begin with the treatment consisted of three minim capsules of wheat germ oil given three times a day, then this was replaced in the later cases by ten minims of the oil given with milk or food three times a day, and ~~in addition by~~ with yeast and gelatin. Finally 4 c.c. of a wheat germ oil-vitamin B mixture was given three or four times a day. This amounted to 2 c.c. of wheat germ oil given each day. The vitamin B seemed to help the vitamin E to act. The appetite improves first, pain on exertion goes, there is a greater resistance to fatigue and finally the rubbery muscles are replaced by tissue of normal consistance. In young patients there is a increase in growth and height and in all, an increased sense of well being. No toxic symptons were noted even if the dose of wheat germ oil was increased to 4 to 6 c.c. daily. The improvement in appetite may have been due to the vitamin B. (Stone. 1940.).

The same Author reports on three cases of pseudohypertrophic muscular dystrophy who improved on treatment with wheat germ oil and the vitamin B complex. One of the cases had almost completely recovered after six months of treatment except for a lordosis. These results would have been of more value if both

vitamins E and B had not been given at the same time. (Stone. Manchester.1941.).

Harris records five cases of progressive muscular dystrophy whom he treated with vitamin E. Four of the cases were male and one female. The first appearance of muscular weakness in this latter case appeared during one of her pregnancies. d - l -α- tocopheral acetate was given orally in doses of nine to two hundred milligrams daily for three to six months. At intervals of one to two weeks during the treatment and after treatment was completed the patient was put on a meat-free diet for two days and after this a twenty-four hour specimen of urine was collected and the creatine - creatinine content estimated. Three of the patients were subjectively better and one showed some objective improvement. Only two of the cases showed a tendency for the creatinine content of the urine to rise, which should occur if improvement is taking place in the muscles. In the other cases there was no change or a slight fall in the content. The diet affects the creatine more than the creatinine so the content of the latter substance in the urine should be a better guide than the former in assessing the results of treatment. As the muscular dystrophies progressed the total quantity of creatinine in the urine decreased, and in spite of the rise in two of the cases the total quantity was still abnormally low. There was also a marked creatinuria in all the cases and this and the low creatinine output seemed to be in keeping with the lack of objective improvement ~~in the cases~~. Two of the cases, who were males, had a family history of the disease. Harris did not think these results were sufficiently marked to draw conclusions from. (Harris.1941.).

Robie records one case of pseudohypertrophic muscular dystrophy which improved in strength under treatment with vitamins E and Bi, and this has been maintained for two months so far. (Robie,1941.).

It may be mentioned here that cases of muscular dystrophy have been found to improve on treatment with vitamin B6 alone. Autopal and Shotland report six such cases, suffering from pseudo-hypertrophic muscular dystrophy. None of the cases were completely cured, and one relapsed in spite of treatment. One of the cases, who was getting progressively worse, improved on receiving vitamin B6, relapsed when saline was substituted for this substance, and improved again when the vitamin was resubstituted. (Autopal. Shotland. 1940a. 1940b.).

Other Authors have found that vitamin E. is of no value in the treatment of muscular dystrophies. Shelden, Butt and Weltman report eight cases of muscular dystrophy who did not respond to treatment with vitamin E. They received three tablespoonfuls of wheat germ oil by mouth with each meal and in most cases were also given a hundred milligrams intramuscuarily of synthetic ∝ tocopheral in sterile peanut oil. Some of the patients received fifty milligrams of ∝ tocopheral by mouth each day. Half of them were treated for over five months and three for only three months; the rest were treated for periods between these two times. All but two of the patients were ambulatory. It was suggested that the treatment may not have been carried out for long enough, or that vitamin E. may not be effective unless certain other vitamins or essential nutrient materials are also present in excess. For these reasons it was not considered that the results should be interpreted as a definite failure of vitamin E. in the therapy of these diseases. (Shelden, Butt Weltmann. 1940.).

The same Authors published a further report after more vigorous treatment of the same cases and of one further case of the same disease. They treated them with at least one tablespoonful of wheat germ oil three times a day along with bile salts by mouth, and gave them a hundred milligrams of synthetic ∝ tocopheral intramuscuarily twice a week in addition. In some cases this treatment was substituted by the administration of wheat germ oil concentrate by mouth to supply two hundred milligrams of tocopheral daily, and by a hundred milligrams of ∝ tocopheral along with a preparation of the vitamin B complex intramuscuarily three times a week, or by two hundred milligrams of synthetic ∝ tocopheral daily by mouth. As a result of these treatments five of the cases showed no improvement, three were subjectively better and one showed a slight increase in strength. Patients who were treated when the disease was in its early stages did not respond better than old standing cases. From these results there seems little evidence that vitamin E. is of benifit to patients suffering from this disease, but it is possible that in these particular cases it was not a significant etiological factor. (Eaton, Weltman Butt. 1941). Fitzgerald and McArdle treated nine cases of muscular dystrophy with vitamin E. with negative results; five of them were suffering from pseudohypertrophic muscular dystrophy, two from Erb's juvenile dystrophy, one from Buzzard's pelvic type and one from a dystrophy which may have been an atypical case of motor neurone disease. In no case, except the last, was the diagnosis in doubt or the disease

too far advanced when treatment was started; so that one would expect to get relief if ~~its~~ the natural history of this disease was to be changed. The patients were four to eleven weeks, with an average of eight weeks, in hospital and were under observation for nine to thirty seven weeks with an average of twenty four weeks. Clinical changes were carefully noted and simple test of muscular power carried out, such as lifting weights and simple exercises. They were observed for about one week before treatment was started and in some cases control periods were instigated during the treatment when inert substances only were given. This was of importance in patients showing subjective improvement and stating that they became worse on withdrawal of the vitamin. therapy Observations were carried out on the creatine and creatinine metabolism. The patients were placed on a low creatine diet, excluding meat, fish and their extracts, and twenty-four hourly specimens of urine were collected each day and estimated for their creatine and creatinine content. The diet was given for a week before treatment was begun, and the patients were weighed at weekly intervals. Crude preparations of vitamin E and synthetic tocopherol were given alone or in combination to the patients, and the dose was varied in the same and different patients. The dose was greater than that used by most workers except Spies and Viltner who gave five hundred milligrams of d-l-α-tocopherol acetate in single doses by injection. The normal dose was eighteen to thirty-six milligrams of tocopherol daily by mouth, but this was sometimes increased and combined with synthetic preparations given intromuscuarily. Some of the cases received vitamin B6 only, or both vitamins B6 and E. The vitamin B6 was given either orally or parentally. On discharge from hospital the patients still continued treatment, either receiving wheat germ, α tocopherol, or d-l-α-tocopherol actetate.

Under this treatment four of the subjects, including the Erb Juvenile forms, felt subjectively better but were not clinically so, and the rest showed no improvement of any kind. The creatine - creatinine metabolism was apparently unaffected by the treatment and no explanation for this was offered by the Authors. Details of the cases will now be given: of the five cases of ~~progressive~~ pseudohypertrophic muscular dystrophy, the first received vitamin B6 subcutaneously and intravenously, and eighteen milligrams of α tocopheral daily, being discharged on this latter substance alone. There was no subjective or objective improvement, in fact the weakness and difficulty in walking, of which the

patient complained, became worse. The second received vitamin B6 alone and became objectively worse. The third was treated with vitamin B6 subcutaneously and intravenously while in hospital and with wheat germ capsules on discharge, but was likewise unaffected. The same result was recorded in the fourth case after treatment with eighteen milligrams of α tocopherol and with vitamin B6; and in the fifth case after treatment with three hundred milligrams of d-l-α-tocopherol acetate intramuscuarily each day for a week, after which a hundred and eight milligrams of α tocopherol orally were added daily; and after another four days, a hundred milligrams of vitamin B6 were also given intravenously each day. This last case was discharged from hospital on a hundred and eight milligrams of α tocopherol. One of the cases of Erb's juvenile dystrophy was only treated with vitamin B6 and showed some subjective improvement. The other recieved thirty-six milligrams of α tocopherol daily for six weeks and then eighteen milligrams for the rest of the treatment and also was subjectively better. The Buzzard types of mascular dystrophy was treated with eighteen milligrams of α tocopherol orally and vitamin B6 subcutaneously while in hospitals, and since discharge has received either eighteen milligrams of α tocopherol or wheat germ capsules daily. There was no objective change but the patient stated that the muscles became less easily tired after treatment. Finally the case of muscular dystrophy, which was difficult to diagnose from a case of motor neurone disease, received three capsules of wheat germ oil daily for a year, each capsule containing 6 ~~m.g.~~ milligrams of α tocopherol, and then thirty-six milligrams of α tocopherol daily for five and half weeks, and from then on eighteen milligrams of α tocopherol and four ounces of Bemax daily. The case was subjectively improved while in hospital but showed no objective change. (Fitzgerald. McArdle. 1941).

Thirteen cases of pseudohypertophic muscular dystrophy were treated with vitamin E by Ferrebee, Kingman, and Frantz with disappointing results. Most of the patients received two table-spoonfuls of wheat germ cereal once a day, and one ten milligram tablet of d-l-dα - tocopherol acetate three times a day or one to two capsules of tocopherex, which each contain forty milligram of d-l-α-tocopherol acetate, twice a day. In addition one to two hundred milligrams of d-l-α-tocopherol acetate in oil were injected intramuscuarily twice a week and vitamin B6, in the form of pirodoxine hydrochloride, was given orally. The treatment was continued for one and a half to fifteen months in various cases,

and in four the disease remained stationary and in one case there was some subjective improvement but most became progressively worse. In two of the cases, in which it was tested for, the creatine content of the urine and the creatine tolerance was unaffected. Biopsy examination of the muscles of six of the patients did not show irreparable changes so the Authors could not give an explanation for their bad results, but suggested that further experiments be carried out with untreated control cases and a more careful analysis of the creatine - creatinine metabolism.(Ferrebee. Kingman. Frantz. 1941.).

Doyle and Merritt report on one case of Erb-Landouzy scapulo - humeral dystrophy and one of pseudohypertrophic muscular dystrophy. They gave the patients a diet rich in fruit, vegetables, eggs, meat, cereals, milk and butter, and for three to four months administered fifteen cubic centimetres of cod liver oil and a dram of vitamin B complex once a day, and twenty-five milligrams of vitamin B1 as thiamin hydrochloride and one cubic centimetre of liver extract intramuscuarily three times a week. Then vitamin E. was added to the treatment in the form of two drams of wheat germ oil orally and sixty milligrams of α tocopherol intramuscuarily once a day. The case of pseudohypertrophic muscular dystrophy was only treated for three weeks but the other case was treated for a longer time. There were no objective signs of improvement, the patients loosing weight and the dynamometric readings in the hand becoming worse.(Doyle. Merritt.1941.).

Bang treated three cases of muscular dystrophy for between one and twelve months with vitamin E.. α tocopherol was given by mouth in daily doses of ninety milligrams, and as a result the condition of one of the cases remained stationary and two became progressively worse. The spontaneous excretion of creatine in these cases did not become less under treatment. The biopsy examination of muscles taken from these patients confirmed the diagnoses and when repeated at a later date showed some signs of the arresting of the degenerative processes; so, although there was no real clinical improvement on treatment with vitamin E., there is some basis for its use in these diseases. (Bang.1941.).

McBryde and Baker report on three cases of pseudohypertrophic muscular dystrophy. The first case was eight years old and was given vitamin B6

intravenously and by mouth and seventeen intramuscuarilar injections of twenty-five milligrams of ∝ tocepheral in thirty six days, but showed no improvement and lost weight. The second case was five years old and received one cubic centimetre of wheat germ oil daily for forty-eight days, but did not improve although there was some subjective improvement and gain in weight after adding vitamin B6 given orally and intravenously. The third case was ten years old and was similarily treated and did not show any improvement even after adding vitamin B6. Other cases of this disease treated with vitamin B6 alone did not improve either. So these results are uniformly negative, the gain of weight in one of the cases being probably due to the general unspecific effect of the vitamin therapy. (McBryde. Baker. 1941).

Seventeen cases of pseudohypertrophic muscular dystrophy were treated with ∝ tocopheral by De Jong and others but showed no response. The Author considered that there was a possibility that the disease might be retarded or arrested by this treatment in certain cases, but admitted that this was purely speculative. There was certainly no benefit in far advanced cases. (De Jong. 1941).

Fleischmann treated two cases of muscular dystrophy with ∝ tocopheral and tesosterone but they showed no clinical improvement. The creatine content of the urine was noted in these cases but there were no significant changes under treatment. (Fleischmann. 1941).

All these results may be tabulated as follows:

Name of Author	Number of cases treated.	Number of cases showing objective improvement.
Bicknell.	18.	12.
Stone.	5.	5.
Stone. Manchester.	3.	3.
Harris.	5.	3.
Robie.	1.	1.
Sheldon. Butt. Waltman. Eaton. Waltman. Butt.	9.	1.
Fitzgerald. McArdle.	7.	0.
Ferrebee. Kingman. Frantz.	13.	0.
Doyle. Merritt.	2.	0.
Bang.	3.	0.
McBryde. Baker.	3.	0.
De Jong.	17.	0.
Fleischmann.	2.	0.
Total number of cases treated and showing objective improvement.	87.	20.

These figures shows that an objective improvement has only been noted in twenty-two percent of cases, and in some of these other treatment has been given in addition to vitamin E, which seems to indicate that the vitamin is of little value in the treatment of these diseases, anyhow once they have started to progress.

2. Motor Neurone Disease.

Motor neurone disease includes cases of amyotrophic lateral sclerosis, progressive muscular atrophy and bulbar paralysis. If reports on these conditions are part of a series of cases that has already been referred to, certain details will not be repeated but a cross reference to the Authors concerned will be given. This applies to following sections as well as to this one.

The more favourable of the reports will be

given first, although none of them give conclusive proof of the efficacy of vitamin E in the treatment of this disease. Bicknall treated four cases of amyotrophic lateral sclerosis with half an ounce of wheat germ twice a day. Two of the patients improved after three and seven months of treatment respectively. In one of these cases, which showed no evidence of upper motor neurone involvement, the mascular fibrillations disappeared and the strength returned in a striking manner. The other two died after four and six weeks of treatment respectively but these cases were complicated by advanced bulbar symptoms. The Author admits that these cases are of insufficient number to draw definite conclusions from, but suggests that they lend support to the theory that this disease is probably due to a nutritional deficiency. (Bicknell.1940-.).

Wechsler notes that this disease has a more insideous onset and lasts one or two years longer if it begins in the legs. The more usual course of events is a loss of power, with muscular atrophy and fibrillations beginning in the upper limbs and spreading to the medulla with fatal results. The clinical picture is one of paralysis and atrophy with increased deep reflexes.

In his first report he describes six cases of amyotrophic lateral sclerosis. The first after not responding to vitamin B1 was treated with whole wheat germ oil and then with two tablets of ephynal, containing three milligrams of synthetic d-l-α-tocopheral acetate, three times a day, and the symptoms improved immediately. The Interossei, thenar and hypothenar muscles filled out and the power of the hands returned. The weakness ~~returned~~ reappeared when the treatment was stopped on two separate occasions. The second case first of all received vitamin B1 and a rich diet for one month and showed slight improvement. Then one tablet of the α tocopheral acetate was added three times a day and there was a striking increase in the muscular strength, the power of swallowing improved and the patient was able to get up and about. After two months of treatment the atrophy and fibrillary tremors of the tongue had disappeared and the patient could walk without assistance. The diet of this patient was practically devoid of green vegetables. Wechsler considered that the relapse on stopping treatment, described in the first case, indicated that the recovery was a direct result of the treatment, although it might have been only a subjective improvement ~~in the first place~~ as the patient knew when the treatment was stopped and

started. He found that the youngest symptoms improved first. The remaining cases did not respond to treatment but were all far advanced cases which would not be expected to improve. (Wechsler, 1940.a).

In his next report the same Author admits that one of the cases reported above has relapsed slightly after the initial improvement, but both are on the whole, progressing satisfactorily although no definite cure can be claimed in either case. As a result of treating twenty further cases of the same disease with vitamin E two recovered, nine improved, in three the disease remained stationary, four became worse and two died. One of the patients who improved was a bedridden woman of thirty-six years of age, and after ten months of treatment she was able to walk and do some housework. The cases who died both suffered from bulbar symptoms and one from carcinoma of the pancreas as well. The treatment consisted of thirty and later fifty milligrams daily of .Ephynal by mouth on an average, although in some cases one hundred to two hundred milligrams were needed. In about half the cases fifty milligrams of tocopheral in oil were given intramascuarily in addition. Every patient received a diet rich in vitamin E. consisting of lettuce, kale, whole wheat bread, course cereals, butter, bananas, fresh corn, fresh peas and beans, the yolk of eggs and fat beef, and also two teaspoonfuls of wheat germ oil and a vitamin B complex preparation daily. Two to five grains of bile salts were given to aid absorption. The lettuce was given in case there was an unknown factor present in it, and not in wheat germ oil, that is needed for the normal function of the nervous system. Two of the cases relapsed on stopping treatment and in three of the cases there was a definite history of previous dietary dificiencies. The Author considers that only certain degenerative types of amyotrophic lateral sclerosis are due to vitamin E. deficiency and can therefore be expected to respond to this treatment, but this question has already been considered. He noted that infections interrupted the recovery when it occured. Seven of the cases were below forty years of age and only seven were over fifty. Most of the cases showed an early occurance of bulbar signs. The excessive salivation seen in cases with bulbar signs may be due to involvement of the salivary nucleus in the medulla, but the loss of voice and difficulty in speech may be due to involvement of the pyramidal tracts only. Wechsler did not know whether oral or parental treatment was best, but it seems that the latter would be, owing to the possibility of defective absorption. (Wechsler .1940.b).

In another series of cases Wechsler treated twelve cases of amyotrophic lateral sclerosis with eight milligrams of ephynal daily by mouth. One case responded remarkably and one showed a slighter recovery of muscular power. These cases relapsed on withdrawal of the vitamin. Three cases showed advanced symptoms when treatment was started and showed no signs of recovery, and the rest appeared to show a remission in the progress of the disease but this was somewhat indefinite. One of the cases may have been wrongly diagnosed. (Wechsler. 1940.c.).

In his last report Wechsler gives the details of the treatment of thirty-six further cases of this disease. Five of them definitely improved. The first case was sixteen years old and the second forty, and in both cases after treatment the muscular atrophy and fibrillation had almost completely disappeared. The third case, aged forty-one, can now walk and so can the fourth. The fifth case showed general improvement. Five other cases did not improve so markedly but seemed to derive some benefit from treatment with vitamin E. Twenty-six of the patients did not improve at all, in fact some, especially those with bulbar symptoms, got worse under treatment. Only four of the cases had a history of taking a diet which might have been deficient in the vitamin. (Wechsler. 1941. a., 1941. b.).

Rosenberger treated nine cases of amyotrophic lateral sclerosis with vitamin E. He used the preparation called ephynal in doses varying from twenty-seven to three hundred milligrams daily and the treatment was continued for several months at a time. A diet with a high vitamin E. content was also given, and better results were observed when vitamin Bi was given before or along with the vitamin E. Five of the cases showed objective improvement, three subjective improvement and one became worse. These results led Rosenberger to believe that vitamin E. does play a part in the etiology of this disease. (Rosenburger. 1941.).

Nine cases of progressive muscular atrophy were treated with Germal E., which is a defatted wheat germ oil preparation, by Mahoney. Three of the cases, who were suffering from the signs of advanced bulbar palsies, did not improve, but the rest showed definite signs of recovery with improvement in strength and vigour. One of these cases suffered from marked wasting of the neck and shoulder muscles, three of wasting of the hand muscles, and two of little muscular wasting but of marked spasticity. All these conditions improved ~~under treatment~~ but the filling in of the hand muscles in two of the cases, suffering from atophy of these, was the most marked change which occured after treatment with the vitamin. The improvement

was first noted forteen to twenty-eight days after the start of the giving vitamin E therapy, and three cases became worse when the administration of the vitamin was stopped, improveing once more when it was started again. The amount of α tocopherol in the preparation of vitamin E used in these experiments each day was twenty-five milligrams. When half this amount was used in one case it was found to be insufficient. (Mahoney. 1941.).

In a paper by Wortis and Jolliffe three cases of amyotrophic lateral sclerosis are quoted who improved after treatment with vitamin E. Treatment was continued for about a year and after that time two of the cases, who showed advanced symptoms, had got no weaker and the atrophy and fibrillations of their muscles had vanished. The other case had only complained of symptoms for ~~the last~~ six months and showed no muscular atrophy, so as would be expected there was a quicker recovery, and after two months of treatment the patient was able to do hard work and the positive Babinski sign and the fibrillary twitchings of the muscles had gone; the only complaint being a cramp-like pain in the calves when fatigued. (Wortis. Jolliffe. 1941.).

Slaughter and Cleckley treated an unusual case of amyotophic lateral sclerosis occurring in a patient only eighteen years old. They gave one dram of wheat germ oil daily for five months which resulted in a steady improvement in the condition of the patient, although an absolute cure was not claimed. (Slaughter. Cleckley. 1941.).

Spies noted that synthetic α tocopherol benefited occasional cases of amyotrophic lateral sclerosis and notes two cases which showed particular improvement. (Spies.1940.)., (Spies. Viltner. 1940.). It may be mentioned that Spies also recorded improvement in patients suffering from this disease after treatment with vitamin B6 alone. (Spies. Hightower. Hubbard.1940.).

For the sake of completeness an amyotrophic lateral sclerosis - like syndrome resulting from the use of sulphathiazole, and prevented by vitamin E, may be described. There was some difficulty in diagnosing the case but it appeared more like a case of amyotrophic lateral sclerosis than one of peripheral neuritis, which was the main alternative. It came on soon after the administration of sulphathiazole given to combat a cellulitis and lymphangitis of the patients left leg, and when one three minim capsules of vitamin E was given three times a day all

the neurological signs and symptoms vanished. They returned when the sulphathiazole was again given without vitamin E., and once more disappeared when both these substances were given together. The patient was suffering from malnutrition, but the Authors could give no explanation for these changes. They suggested that vitamin E and sulphathiazole might be used together in certain cases of infective involvement of the nervous system. (Weinberg. Knoll. 1940.).

The remaining reports on the treatment of this disease with vitamin E are almost entirely negative. Worster-Drought and Shaffer treated twenty-five cases of progressive muscular atrophy, amyotrophic lateral sclerosis, and Bulbar paralysis for five to ten months with eighteen to thirty milligrams of α tocopherol acetate daily with disappointing results. They note the difficulty of sometimes diagnosing cases of amyotrophic lateral sclerosis from ones of disseminated sclerosis, and only diagnose a case as suffering from the former disease if there are no remissions in its course, it starts after middle age, and exhibits no symptoms of incordination. In all the cases the cerebro-spinal fluid was normal, there were no lesions of other systems and the Wassermann reaction was negative. There were nine cases of bulbar paralysis, six female and three male, and most of them had spinal motor neurone degeneration as well. Eight of the patients received thirty milligrams of α tocopherol acetate daily for periods varying from six to nine months, and one wheat germ oil, which was the equivalent of eighteen milligrams of α tocopherol a day, for ten months. Three of these cases died after eight months treatment and the rest became steadily worse, although one case showed some subjective improvement. Nine of the patients suffered from progressive muscular atrophy although four of them showed symptoms of upper motor neurone involvement as well. Six of them were males and three females and they were aged from forty-two to fifty-nine years. All except one were treated for periods varying from six to nine months with six milligram capsules of α tocopherol three times a day, and as a result the disease process advanced in five of the cases, there was no change in two of them after five to six months of treatment and two showed some improvement in their gate and general health. One of the cases whose nervous condition remained stationary felt more cheerful. The remaining seven cases cases were ones of amyotrophic lateral sclerosis, six of them were male and one female and their ages varied from forty-five to fifty-two years. They were treated

for periods varying from six to nine months and as a result of this five patients became worse, one showed an advance in the atrophy of his muscles but his general health and mental outlook was improved, and two definitely improved. These last two cases showed an improvement in the power of the limb muscles and in the ability to walk. The muscular wasting in the hands was less marked, the general health was better, and they were more cheerful. The case which improved subjectively could walk better after five months of treatment although there was no change in the physical signs, but this was probably not due to vitamin E. but to an increased effort to recover on the part of the patient. All the cases suffering from amyotrophic lateral sclerosis received thirty milligrams of α tocopherol a day. The unexpected feature of these results is the marked effect on the general vitality and emotional tone in several of the cases. The treatment seemed to mitigate the dispairing and depressing outlook on the disease, even if the patients were getting worse, and in two cases this even amounted to euphoria. This effect is more likely to be a psychological one than to be due to vitamin E. (Worster.— Drought. Shaffer. 1941.).

Veits, Trowbridge and Gunderson treated seventeen cases of amyotrophic lateral sclerosis and six of progressive muscular atrophy with vitamin E. The patients were given wheat germ oil and α tocopherol acetate, starting with five to ten milligrams per day of the latter substance, the dose of which was eventually increased to from two hundred to three hundred milligrams a day and even up to four hundred and fifty milligrams a day in one case. The patients diagnosed as cases of amyotrophic lateral sclerosis were treated for ten months, six of them out of hospital, and the result was that five died, three became worse, the condition of seven was unchanged, and two showed improvement in their muscular strength and their fibrillary tremors were less marked. Of the patients suffering from progressive muscular atrophy one died, three became worse and the symptoms of two remained stationary after treatment. The Authors considered that the only result of therapy with vitamin E was possibly to slightly retard the progress of the disease. (Veits. Trowbridge. Gunderson. 1941.).

Twelve cases of amyotrophic lateral sclerosis were reported on by Davison. He treated them with fifteen milligrams of vitamin E each day by mouth, and also gave it intramuscuarily. The treatment lasted for two to four months but only resulted in objective improvement in one case with a partial disappearance of the muscular fibrillations. Some of the cases showed subjective improvement but most of them became

worse and four died. The only explanation for these bad results was that most of the cases were advanced bulbar ones. (Davison.1941.).

Bang treated eight cases of amyotrophic lateral sclerosis for periods varying from one to twelve months with vitamin E. In one case injections of synthetic α tocopherol were given and in the others α tocopherol was given by mouth in doses of ninety milligrams. Improvement was noted in one atypical case, the symptoms of two cases remained unaltered and the rest became worse. In six cases there was a moderate spontaneous creatinurea. In one case the secretion of creatine diminished, and in two it ceased all together, during the period of treatment, although the clinical condition of these last two patients did not improve. Samples of the patient's muscles were examined after biopsy and the diagnosis confirmed, and when this was repeated in two cases after two and a half months the vacuolated degeneration of the muscle fibres was found to have ceased. This seemed to indicate that although no marked improvement, after the treatment of this disease with vitamin E, could be expected, there was some basis for the belief that the vitamin has an effect on its progress. (Bang.1941.).

Four cases of progressive muscular atrophy and three of amyotrophic lateral sclerosis were treated by Vivanco for two months with two millilitres of wheat germ oil and forty grams of dried Brewer's yeast daily. Only three did not improve and they were, one patient suffering from progressive muscular atrophy, and two from amyotrophic lateral sclerosis. However this was only a subjective improvement as was proved by a study of the creatine - creatinine excretion. During treatment the creatine excretion did not fall as it should have, but actually rose in all cases, except in one which showed no creatine secretion in the urine during the period of observation, and in one in which no estimation of the creatine content was made. This pointed to a true deterioration in the patients' condition in spite of the treatment with vitamin E. (Vivanco. 1940.).

Sheldon, Butt and Woltman report on six cases of amyotrophic lateral sclerosis and four of progressive muscular atrophy. They received three tablespoonful of wheat germ oil with each meal and most of the cases were also injected intramuscuarily with a hundred milligrams of synthetic and α tocopherol twice a week. Some also took fifty milligrams of α tocopherol daily by mouth. The period of treatment varied from three to five months and all except two of the patients were ambulatory when

this was started. There was no objective improvement in any case; one of the cases of progressive muscular atrophy was subjectively better; and three of the cases of amyotrophic lateral sclerosis were worse. The treatment may not have been carried out for long enough, or some substance other than vitamin E may be also needed for the cure of these diseases. (Shelden. Butt. Woltman, 1940.).

These cases received further treatment and were reported on at a later date, with the addition of five more cases of amyotrophic lateral sclerosis and one of progressive muscular atrophy. They were treated with at least one tablespoonful of wheat germ oil, and also with bile salts, three times a day. Twice a week they received intramuscular injections of a hundred milligrams of synthetic α tocopherol. In a few cases this treatment was replaced either by sufficient wheat germ oil concentrate by mouth to supply two hundred milligrams of α tocopherol daily and intramuscular injections of a hundred milligrams of synthetic α tocopherol three times a week, or by two hundred milligrams of synthetic α tocopherol daily by mouth. A preparation of the vitamin B complex was also given to some of the patients. Five of the patients suffering from amyotrophic lateral sclerosis became worse, five died and in one the signs and symptoms remained unaltered. Two of the cases of progressive muscular atrophy died, one became worse and the condition of two remained almost stationary. Early cases did not respond better than more advanced ones. These results seem to indicate that vitamin E was not an etiological factor in this series of cases anyhow. (Eaton. Woltman. Butt. 1941.).

Eight cases of progressive muscular atrophy, bulbar paralysis and amyotrophic lateral sclerosis were treated with vitamin E by Doyle and Merritt. They received a rich diet of fruit, vegetables, eggs, meat, cereals, milk and butter, and also cod-liver-oil, vitamin B complex and liver extracts for three to four months. Then vitamin E was added to the treatment in the form of two drams of wheat germ oil by mouth and sixty milligrams of α tocopherol intramuscularly each day. A month later vitamin B6 was also given. This treatment varied slightly in some cases. It lasted from three weeks to eleven months in various cases, being usually of several months duration. In spite of this treatment nearly every case lost weight and the dynamometric readings in the hand became worse. Two early cases of amyotrophic lateral sclerosis progressed rapidly and none of them showed

objective signs of improvement, although several said they felt better which may have been due to the special diet. Only one case, suffering from amyotrophic lateral sclerosis, had been previously taking an inadequate diet. (Doyle. Merritt. 1941.).

Ten cases of motor neurone disease are reported on by Fitzgerald and McArdle. They were treated while in hospital with an average dose of eighteen to thirty-six milligrams of tocopherol daily, although this might be increased to as much as a hundred and eight milligrams in some cases, and combined with three hundred milligrams of d-l-α-tocopherol acetate, and with vitamin B6. On discharge from hospital the patients continued to take either wheat germ, α tocopherol or d-l-α tocopherol. No improvement of the patients' condition and no alteration in the creatine-creatinine metabolism was noted as a result of this treatment. Two of the cases died while under treatment with α tocopherol, their muscular weakness increasing, and the new signs and symptoms of fibrillary tremors of the tongue and dysphagia starting before this occured. Two other cases have since been admitted to other hospitals in a deteriorated condition and one case, which showed no change while in hospital, has since developed an increased muscular weakness and more marked muscular fibrillations. Three cases did improve subjectively, but after a while even one of these became much worse. The remaining cases steadily deteriorated subjectively and objectively. Further details of these cases will be given. The first case received eighteen milligrams of α tocopherol daily for two weeks and then thirty-six milligrams daily for six weeks. Fibrillation of the tongue started while in hospital and death occured soon after discharge. The second case was treated with thirty-six milligrams of α tocopherol daily but dysphagia developed and death occured while still under treatment. The third case showed no objective or subjective improvement after treatment with thirty-six milligrams of α tocopherol daily, in fact the muscular strength steadily deteriorated. The fifth case received thirty-six milligrams of α tocopherol daily for seven weeks and then eighteen milligrams each day. There was no subjective or objective improvement and the case has since been admitted to another hospital. The sixth case showed a slight subjective improvement after treatment with eighteen milligrams of α tocopherol daily and with massage. The seventh case received eighteen milligrams of α tocopherol daily for three weeks, thirty six milligrams daily for a week, and then eighteen milligrams daily once more. There was some subjective improvement but the patient was objectively worse Eighteen milligram of α tocopherol were given daily for four weeks to the eighth case and this was then increased to thirty-six milligrams daily for two

weeks, and after that to a hundred and eight milligrams daily for another two weeks. Finally the dose was reduced again to eighteen milligrams a day. In spite of this treatment there was no subjective or objective improvement and the patient has since become bed-ridden and been admitted to another hospital. The ninth case received thirty-six milligrams of α tocopherol daily for one and a half weeks and then a hundred and eight milligrams daily for six weeks till the time of discharge from hospital, when the dose was reduced to twenty-four milligrams a day. There was no subjective or objective improvement of the signs or symptoms of the disease observed. The last case was treated with thirty-six milligrams of α tocopherol daily for three weeks, a hundred and eight milligrams daily for the next ten days and then inert capsules were substituted for the vitamin for two and a half weeks. So far the vitamin E had always been given by mouth but, after the treatment with inert capsules, three hundred milligrams of d-l-α- tocopherol acetate were given intramuscuarily along with a hundred and eight milligrams of α tocopherol by mouth each day for three days; vitamin B6 was given subcutaneously and intravenously, in addition to similar quantities of the d-l-α- tocopherol acetate and the α tocopherol, daily for the next six days; and finally for the last seven days of the treatment inert capsules were given by mouth and saline injections given intramuscuarily and intravenously. No objective improvement was ever observed throughout this treatment, but the patient became subjectively better as soon as the treatment was started and this was maintained till ten days after the beginning of the first treatment with inert capsules. Then the patient began to feel more tired and became more conscious of twitchings in the legs. Personal worries at this stage may have contributed to this deterioration. Subjective improvement again occured when the treatment with α tocopherol and d-l-α- tocopherol acetate was started. The lack of objective improvement in this case indicates that any effect the vitamin appeared to have was probably psychological, the case was unaffected by the treatment with vitamin B6, although on one occasion there was a dramatic subjective improvement within an hour of giving a hundred milligrams of vitamin B6 intravenously. A similar improvement occured after an intravenous injection of saline next day. (Fitzgerald. McArdle. 1941).

Ferrebee, Klingman and Franz treated six cases of amyotrophic lateral sclerosis and seven of progressive muscular atrophy with vitamin E. Most of the patients received two tablespoonfuls of wheat germ cereal a day, and one ten milligram

tablet of d-l-α- tocopherol acetate three times a day. Others were treated with one to two capsules of tocopherex, containing forty milligrams of d-l-α-tocopherol acetate, twice a day, and with intramuscular injections of one to two hundred milligrams of d-l-α- tocopherol acetate in oil once or twice a week. Vitamin B6 was also given to some patients. After this treatment which lasted from six to twelve months, no improvement was observed in any of the patients although six of those suffering from progressive muscular dystrophy became no worse. (Ferrebee. Klingman. Frantz. 1941).

Eleven cases of amyotrophic lateral sclerosis, one of which was in its very early stages, were reported on by Denker and Scheinman. One died in three weeks but the rest were treated for at least one month. Most of the patients were treated with vitamin E both orally and parentally so that they received fifty-five to a hundred and seventy five milligrams of α tocopherol each day. Ephynal, a preparation of synthetic d-l-α- tocopherol acetate, was given by mouth, and an oily solution of tocopherol containing five milligrams per cubic centimetre was given intramuscuarily. No toxic results were observed. There was a slight subjective improvement in some cases at the onset of the treatment but this was not maintained. Two of the patients died of concurrant broncho-pneumonia, two became markedly worse, and the condition of the rest slowly but surely deteriorated. None of the cases gave a history of dietory deficiencies or of gastro-intestinal symptoms, and one was a vegetarian who should have been sure of an adequate supply of vitamin E. The same Authors treated four more cases of amyotrophic lateral sclerosis at a later date with two hundred milligrams of α tocopherol daily and also with vitamin B6, but recorded no improvement in these patients either. (Denker. Scheinman. 1941.).

Finally in a paper, written by De Jong, further cases of amyotrophic lateral sclerosis treated with vitamin E are cited. In the first series there are twenty cases, one of which was also suffering from syphilis. They showed no response to treatment with α tocopherol except a slight decrease in the fibrillary tremors of the muscles in some cases, although several of the patients said that their general health felt better. In the second series there were eleven cases of amyotrophic lateral sclerosis and six of progressive muscular atrophy and in the third series there were twenty one cases of amyotrophic lateral sclerosis. None of these showed any improvement on treatment with vitamin E. The Author thought that vitamin E was certainly of no value in the advanced

stages of these diseases, but considered that it was justifiable to carry out further investigations. (De Jong.).

All these results may be tabulated:

Name of Author.	Number of cases treated.	Number of cases showing objective improvement.
Bicknell.	4.	2.
Wechsler. 1.	6.	2.
2.	20.	11.
3.	12.	2.
4.	36.	10.
Rosenberger.	9.	5.
Mahoney.	9.	6.
Wortis. Jolliffe.	3.	3.
Slaughter. Cleckley.	1.	1.
Spies.	2.	2.
Weinberg. Knoll	1.	1.
Worster. Drought. Shaffer. Veits. Trowbridge.	25.	4.
Gunderson.	17.	2.
Davison.	12.	1.
Bang.	8.	1.
Vivanco.	7.	10.
Sheldon. Butt. Woltman.; Eaton.		
Woltman. Butt.	16.	0.
Doyle. Merritt.	8.	0.
Fitzgerald. McArdle.	10.	0.
Ferrebee. Klingman. Frantz.	13.	0.
Denker. Scheinman.	15.	0.
De Jong.	52.	0.
Total number of cases treated and of cases showing objective improvement.	286.	53.

From these figures it is evident that only nineteen per cent of cases of motor neurone disease treated with vitamin E preparations have been reported to show an objective improvement. Many of these cases have been treated with other substances as well as with vitamin E which lessens the value of the results still further; and until some definite citereon of improvement

is fixed upon in the treatment of these diseases, it is difficult to compare the results of different Investigators. The effect of the therapy in question on the creatine - creatinine metabolism, and the excretion of these substances in the urine, seems to supply this need, but few of these Authors have carried out such estimations. Those that have report that vitamin E has practically no effect on their excretion. This fact, and the percentage of improved cases given above, for what value it is, do seem to indicate that vitamin E can be of little use in the treatment of this disease. This is what experimental data would lead you to expect, as even in laboratory animals, the chief rôle of the vitamin is in the prophylaxis of pathological conditions of their nervous systems, which may be compared to motor neurone disease in man.

3. TABES DORSALIS.

Stone treated eighteen cases of tabes dorsalis with vitamins B and E for periods varying from three months to two years. After this the patients showed an improvement in their gait, in their muscular strength, tone and co-ordination, and in their bladder function. The gastric crises and lightening pains also came on less frequently. In one case symptoms like those of amyotrophic lateral sclerosis were rapidly relieved and regeneration of the muscles occurred. Most of the cases also received treatment with arsenic and artificial pyrexia, so the vitamin may only increase the patient's tolerance to arsenic or enhance the results of the fever. However, it has already been mentioned that the effects of vitamin E deficiency may only appear in tissues damaged by another agent, which in this case would be the syphilitic spirochaete: so that apart from killing this organism, it might be necessary to give vitamin E also, in order that its neurotrophic factor could restore normal myelinisation of the tracts of the central nervous system. On the other hand there is rarely any evidence of dietary deficiencies in patients suffering from this disease, and when there is, the absence of some other factor than a vitamin may be causing a detrimental effect on the disease process. The course of the disease is insidious, so claims for any therapy must be made with caution. (Stone.1942.).

Two cases of this disease were treated by Bicknell with half an ounce of wheat germ and showed no improvement as a result. Both cases were in the advanced stages of the disease. (Bicknell.1940.).

An insufficient number of cases have been treated with vitamin E for any definite conclusions to be drawn as to its efficacy in their treatment.

4. SUBACUTE COMBINED DEGENERATION.

Shute has treated cases of myelopathy following pernicious anaemia with vitamin E and reports favourable results. No details of the patient's history are given. (Shute.1939.).

5. Poliomyelitis.

The muscular atrophy which follows attacks of poliomyelitis has been treated with vitamin E with successful results in some cases. Stone treated a case of this nature with wheat germ oil in quantities up to six cubic centimetres, and also with vitamin B, and found that the strength of the muscles increased. (Stone.1940.) . De Jong described no improvement after the treatment of the disease with α tocopherol. (De Jong.1941.).

6. NEURITIS.

The muscular atrophy of patients suffering from multiple neuritis has also been treated with this vitamin. Stone treated one case with wheat germ oil and vitamin B with a resulting improvement in the condition of the muscles. The improvement was most striking when vitamins E and B were given together which led the Author to believe that vitamin B increases the activity of vitamin E. (Stone. 1940). Peripheral neuritis, caused by arsenic, has been found to respond favourably to treatment with vitamin E in some cases. Partial remission of the neuritis, which was of severe type, occurred after giving synthetic vitamin B6, but the remission was more spectacular when vitamins B6 and E were given together. Vitamin E was administered in the form of injections of fifty milligrams of α tocopherol intramuscuarily twice a day for a week. Both the vitamins had to be given daily to maintain the recovery, and if vitamin B6 was withdrawn, it stopped altogether. Vitamin B1 had no effect on the condition. (Vilter. Aring. Spies, 1940).

7. FIBROSITIS.

Steinberg treated eighty-two patients suffering from fibrositis with two to eight cubic centimetres of wheat germ oil three times a day. Thirty of them were classified as cases of primary fibrositis, twenty as fibrositis secondary to atrophic arthritis, twenty secondary to hypertrophic arthritis, one secondary to gout, three secondary to sciatica and eight of the cases as neurotics. All the patients were observed for periods ranging from three months to two years before treatment was started. The cases of primary fibrositis received two to eight cubic centimetres of wheat germ oil three times a day and all their symptoms vanished. Two of the

cases showed a tendency to relapse after four weeks, but this was completely cured after a week's treatment with a quantity of vitamin E molecular distillate each day, which contained a hundred and twenty milligrams of α tocopherol. The patients with fibrositis and atrophic arthritis received the same quantities of wheat germ oil for two to four months and as a result eight improved but twelve were unaffected. On treatment with two to six cubic centimetres of wheat germ oil for two to six months none of the cases secondary to hypertrophic arthritis were improved. Those secondary to gout and sciatica were also unaffected after four weeks' treatment with four cubic centimetres of wheat germ oil a day. The eight neurotics were not relieved of their symptoms after two to three months of treatment with wheat germ oil, although they were helped by barbiturates and bromides. These results led Steinberg to consider that vitamin E was of value in the treatment of primary fibrositis, although concentrated preparations might be needed in serious cases. It seemed to be of little value in secondary fibrositis, so the primary condition may be a metabolic rather than an infective process. These results would need to be confirmed before definite conclusions could be drawn on this question.

8. Calcification of Tendinoscapular Tissue.

Thirty-four patients suffering from this disease were treated with vitamin E by Sutro and Cohen. Twenty-one of them were acute cases and thirteen chronic. Fouteen were males and twenty females, and their ages varied from thirty-three to sixty eight years. There was X Ray evidence of calcification in every case and sometimes this was not confined to the tendinoscapular tissue. Two of the patients had a history of trauma and nine of dietary deficiency. Eight of the acute cases received no exercises, physical therapy or immobilisation of the affected part, but were treated with α tocopherol acetate given orally. Four of them received five to twenty milligrams daily for twenty to fifty-one days, and the other four, fifty to a hundred milligrams daily for seven to twenty-one days.

Thirteen of the acute cases were kept as controls and treated with codeine sulphate, lactose, vitamin B6 and procaine hydrochloride. In the group of patients treated with vitamin E the pain subsided in five to twenty days and active motion was started after periods varying from three to five days after the onset of treatment. The normal range of movement was reached in twenty to fifty days. On X ray examination it was found that the signs of calcification has disappeared in two cases after thirteen and twenty days of treatment respectively. A marked diminution in the size of the calcified areas was seen in another two after thrity and sixty days of treatment and the remaining cases showed a similar picture although it was of slighter degree. Two of the cases showed calcification in the tendinous scapular tissue of both sides although only one side was painful. Under treatment with vitamin E the calcification in the painless shoulder disappeared in eleven days in one case and was diminished in size after four weeks in the other. In the control group the acute symptoms sometimes subsided in the first three days of treatment and disappeared altogether in seven to fifty-five days, and active movements might be started after two to eight days. In six of the thirteen cases the signs of calcification on X ray examination had disappeared after seven to seventy seven days of treatment, and in three the calcified area had diminished in size after eight to twenty-four days. The rest showed no change.

The chronic cases had suffered from the disease for periods varying from one month to twenty years, and had had previous treatment of some sort or another. Eight of these cases received α tocopherol by mouth in doses of ten to a hundred milligrams a day for seven to a hundred days. The total dose varied from two hundred and thirty-one to two thousand, two hundred and fifty milligrams. The remaining patients of this group were kept as controls and treated with diathermy, infra-red rays, exercises and general medication. The group treated with ~~the~~ vitamin E all improved subjectively in twenty to sixty days after the start of treatment. On X ray examination only very slight improvement was noted. Some of these cases also showed signs of calcification in one of their shoulders, which was unaccompanied by symptoms, but in this series it was unaffected by vitamin E. Three of the five control patients improved in twenty to sixty days. The calcification appeared less on X ray examination in one patient, who had only been receiving asperin. Calcification unaccompanied by pain was unaffected. The Authors considered that the pain of the acute phase is a complication, and

the treatment for the pain does not necessarily influence the fundamental condition, and they noted that the calcification might disappear whether the treatment was given or not. They stipulate that the fundamental change in the tissues may be due to vitamin E deficiency, and it may cause an acutely painful inflammatory reaction by a rupture of the degenerated tissues into a bursa or tendon sheath. This reaction may be the cause of the spontaneous absorption of calcium which sometimes occurs. These results seem to indicate that vitamin E may be of value in the treatment of acute cases; preventing further necrosis of tissue and calcification of that already necrosed. It appears to be of no value in chronic cases as it does not alter the clinical picture, or the size of the X ray shadow of the calcified area, to any significant degree.

(Sutro. Cohen, 1941).

9. MALNUTRITION AND GENERAL MUSCULAR WEAKNESS.

Vitamin E seems to be of value in the treatment of cases of Malnutrition. Spies, Hightower and Hubbard report that five hundred milligrams of α tocopherol in oil given by intramuscular injection were effective in relieving muscular weakness of the legs, burning of the soles, roaring in the ears, anorexia and insomnia in fourteen patients suffering from malnutrition. They also exhibited the signs of coarse muscular tremors, diminished vibration sense in the legs, absent ankle jerks, and a mild degree of hyperaesthesia of the skin of the legs. These signs were improved by the treatment in some cases. None of the cases showed evidence of pellagra, beri-beri, or Tibotflavine deficiency. The improvement may only have been a subjective one, as no control observations were carried out on other patients suffering from a similar condition and not receiving vitamin E. In twelve of the cases improvement was marked within twenty-four hours of receiving the treatment, and the patients were able to perform hard work. Their muscular cramps, and tenderness, the burning of the soles and the roaring in the ears diminished; they were able to get employment and so afford a better diet; and finally were completely cured. Four of these cases relapsed, but improved again after a second injection of the same dose of α tocopherol. Two of the patients were never completely cured.

(Spies, Hightower, Hubbard. 1940).
(Spies. Viltner. 1940).

Stone and Manchester found that vitamin E gave a more rapid improvement in cases of general muscular weakness than either vitamin B or orthopaedic means. Flaccid types responded better than spastic. The cases were treated with from eight to sixty minims of wheat germ oil a day and the dose was well tolerated. In very rare cases a reddish papulomacular rash of the skin was observed during the treatment, but this was probably due to impurities in the oil and not to the vitamin E.

(Stone. Manchester.1941).

It may be mentioned that cases of malnutrition, complaining of instability, nervous weakness and awkwardness in walking, have been improved with vitamin B6 alone, although this has not been confirmed.

(Spies. Bean. Aske. 1939).

10. AMYOTONIA CONGENITA.

Several Authors have treated this disease with vitamin E, but with disappointing results. Doyle and Merritt treated one case with wheat germ oil for several months but the course of the disease was unaffected.

(Doyle. Merritt, 1941).

Fitzgerald and McArdle report on one patient whom they treated with eighteen milligrams of α tocopherol daily for two weeks, and then thirty-six milligrams daily for three weeks, finally returning to the first dose till the end of the treatment. The patient was subjectively and objectively worse on the α tocopherol, but did improve when half a grain of ephedrine was added to the treatment each day. The extent of the improvement was unaffected by the vitamin E.

(Fitzgerald. McArdle, 1941).

Fleischmann found no improvement in the clinical state, or in the creatine content of the urine, in one case of this disease whom he treated with α tocopherol.

(Fleischmann.1941).

Bicknell on the other hand treated a patient with this disease with half an ounce of wheat germ, and with massage, daily for three months, and noted a great improvement, which definitely became slower when the administration of the vitamin was stopped.

(Bicknell.1940).

A sufficient number of cases have not been reported on yet to enable the value of the vitamin in the treatment of this disease to be assessed.

11. MYOTONIA ATROPHICA.

Vivanco treated a case of this disease with two millilitres of wheat germ oil, and with Brewer's yeast, daily for two months. There was some subjective improvement, but this was not confirmed objectively.

(Vivanco. 1940).

12. PERONEAL MUSCULAR ATROPHY.

There have been several reports on the treatment of this disease with Vitamin E. Bicknell treated two cases with half an ounce of wheat germ daily for two months. He found there was no improvement as a result of this, but he considers the treatment should have been carried on for a longer time as it is a slowly progressing disease.

(Bicknell. 1940).

Eaton, Woltman, and Butt treated one of these cases, and also one of localised pan-atrophy, with wheat germ oil and synthetic ~~and~~ α tocopherol, but both were unaffected by the treatment.

(Eaton, Woltman, ~~and~~ Butt. 1941).

Doyle and Merritt also treated one case with both these substances and with similar results.

(Doyle and Merritt. 1941).

Four cases of this disease were treated by Veits, Trowbridge and Gunderson with twenty to two hundred and fifty milligrams of synthetic vitamin E each day for eight to thirty six weeks. Some of the cases were also given fifty milligrams of the same substance intramuscuarily three times a week. In spite of this treatment there was no improvement in the condition of any of the cases, although the general health of one or two was better.

(Veits, Trowbridge. ~~and~~ Gunderson. 1942).

13. SPINAL MUSCULAR ATROPHY OF INFANCY.

It has been noted that the lack of vitamin E in the diet of the mother might result in a muscular dystrophy occurring in her child several years after its birth. For this reason Hilden advised the treatment of all cases of spinal muscular atrophy of infancy with vitamin E, and also possibly with vitamin B to enhance the effect of the former, in ~~the~~ case the lack of vitamin E during the early development of the child in **utero** has been an etiological factor. Hilden considers that, as well as the Werdnig Hoffmann type, Oppenhiem's amyotonia congenita may belong to this class of disease.

(Hilden.1941).

14. LORDOSIS.

Donovan successfully treated with vitamin E patients with Lordosis, probably due to a mild degree of muscular dystrophy. He reports on a family in which the Father, Son and Daughter suffered from Lordosis; the son showing definite weakness of the muscles of the proximal part of the limbs and the shoulders, and winging of the scapulae. The mother had not been pregnant for the last ten years although no means of contraception ~~had~~ have been used. Their diet consisted of white bread, imported butter, tea, milk, and sugar for breakfast; a small quantity of imported meat, chipped potatoes, and rice pudding or custard for lunch; and bread, jam, tea and milk for tea. No green vegatables were ever taken. The children sometimes received milk at school. It is evident that this diet is deficient in foods which are rich in vitamin E. Three-quarters of an ounce of whole wheat germ were given four times a day for six weeks. The Father felt better, although his Lordosis was unaffected as might be expected in a condition that had been a long standing one. The Mother was more energetic. The Son walked farther and more freely than before. His lips were firmer and the Lordosis was less marked. He developed a slight urticarial rash while under treatment. The Daughter was more energetic and brighter, her Lordosis completely disappeared and the pains in the limbs, of which she had previously complained, were cured. These results led the Author to consider that some cases of Lordosis were due to mild forms of muscular dystrophy, and therefore amenable to treatment with vitamin E; although the wheat germ oil also contains vitamins B1 and B6 so these may also play a part in the cure.

He also states that it is reasonable to give wheat germ oil prophylactically to children whose own diet, and that of their Mothers, has been of a poor variety.

(Donovan, 1940).

15. MYOTONIA CONGENITA.

Two cases of this disease were treated by Stone and Manchester with wheat germ oil and vitamin B. He reported that both the patients became stronger and developed new muscular tissue.

(Stone. Manchester, 1941).

16. PINK DISEASE.

Forsyth treated three children suffering from this disease with wheat germ. The first case was given one ounce of the wheat germ three times a day. Photophobia disappeared in a few days and the patient began to take notice and return to bright ways within a week. Eating and sleeping became normal, and after a fortnight the skin showed no lesions of any kind. There was an immediate gain in weight. The second case was first treated with one ounce of wheat germ and then one capsule of wheat germ oil, three times a day. This change was made because the child was unable to eat the wheat germ, but improvement was so marked that wheat germ was soon able to be given again. The child also received vitamin B. The third case was similarly treated with wheat germ and wheat germ oil, but developed a dusky cyanosis which vanished when the dose of these two substances was successfully reduced. Six other cases previously treated with vitamins E and B were also mentioned. No explanation of why vitamins E or B should cure this disease was given, and it is impossible to draw conclusions from these uncontrolled results as the disease may clear up spontaneously at any time.

(Forsyth. 1941).

17. MISCELLANEOUS DISEASES.

Patients suffering from Myasthenia gravis, post-encephalytic parkinsonism and paralysis agitans have been treated with vitamin E, but no successes, as a result of this treatment, have been recorded, and no details of the cases given. It may be mentioned that these diseases, and ideopathic epilepsy as well, have been successfully treated with vitamin B6 by some Authors.

(Spies. Hightower. Hubbard. 1940).
(Jolliffe. 1940).

The Summary and Discussion.

The experimental and theoretical data on the actions and uses of Vitamin E. are so conflicting, and in many cases so indefinite, that little more can be done than to point out what has been the most valuable of the experimental work and what are the most likely of the theories brought forward to explain the possible effect of its lack. Points that have already been discussed will only be referred to in in this section with an indication as to where this has been done.

A short history of the vitamin is given to start with. Then the chemical isolation of the vitamin is traced from when an unknown factor influencing normal reproduction was first thought to be present in wheat germ oil till the vitamin was synthesised. Details of the chemical tests are given and it is noted that Emmerie and Engel's method is the best. The test may be applied to estimations of the Vitamin E. content of the blood serum. Methods of biological assay of the vitamin are discussed and the difficulties in performing this are noted. There does not seem to be a very close agreement between the two methods for estimating quantities of Vitamin E, but if it was proved both by chemical tests and biological assay that synthetic tocopherol and natural Vitamin E had similar effects on experimental animals, it would be possible to dispense with the latter test and use only the former. Little information on the action of Vitamin E is derived from the chemical studies except that it is unlikely to play a part in the oxidation-reduction processes of the cell, as when tocopherol is oxidised it gives rise to physiologically inert substances.

The next section deals with certain physiological problems. It is noted that defects in absorption in individual animals and human beings may explain why conditions stated to be due to deficiency of the Vitamin may arise only in certain cases while others remain normal on the same diet. These individual variations may also apply to the utilisation of the vitamin. Examples of diets used to produce Vitamin E. deficiency in experimental animals are given and the evidence for the occurence of a natural deficiency of Vitamin E. in animal and human diets is reviewed. The occurrence of the vitamin in nature is so widespread that this seems unlikely although the question cannot be settled till the absolute quantity of the vitamin in various diets is estimated and till it is known what are the minimal requirements for the vitamin, if any, needed by various animals and by man.

As these requirements are not known the dose of the vitamin needed to cure pathological states due to deficiency of the vitamin can only be estimated by the effect that various doses have in curing these

conditions. There is no doubt that lack of the vitamin caused disturbances of the reproductive and nervous systems of rats, but it is not so certain that similar conditions in human beings are also caused by this deficiency. So in the present state of our knowledge the only way to estimate the curative dose in man, is to find the amount of the vitamin which cures a similar condition in the rat, and calculate it by means of relative weights. This is obviously unsatisfactory but till it is known whether the vitamin is needed by man at all, it would be better to have a standard scale of dosage so that the work of various investigators could be more easily compared. A list of the proprietary preparations used in clinical medicine is given and in most cases the amount of tocopherol that these contain is stated.

The fact that little storage of the vitamin occurs in the body is noted. The storage that does occur, takes place mainly in the subcutaneous and intraperitoneal fat. The means of supply of the vitamin from the mother to the young in experimental animals is described. It takes place almost entirely through the mammary gland and very little through the placenta. That the supply is small in amount is proved by the fact that it is insufficient in females to prevent resorption in the first gestation or to protect males from testicular damage for more than seventy to eighty days.

Excess of the vitamin apparently produces no harmful effects but certain cases of hypersensitivity to the vitamin are quoted. It is not quite certain if these were really due to the vitamin itself or to other impurities in the preparations used.

Finally it is noted that excretion of the vitamin only occurs when the intake is high and that excretion in the faeces occurs at a lower intake than excretion in the urine.

After these introductory sections a study is made of the results of a deficiency of the vitamin on the various systems of man and of animals. First the effect of Vitamin E deficiency on cell metabolism is considered. It is thought that the vitamin may be needed for the normal function of the cell nucleus and that lack of it may result in an interference with cell division. It may act as a morphological hormone necessary for synthesis of the nuclear chromatin or for its physio-chemical structure. Certainly if this was so it would offer a common etiological cause for all the effects of Vitamin E deficiency, the occurence of lesions in certain situations such as the nervous system, the developing embryo and the germinal layers of the testis, being an acute manifestation of the deficiency in cells which are rapidly dividing or have been previously damaged by some other noxious agent. Changes in the cytoplasm of these cells are almost certainly

secondary to nuclear changes. Against this theory of restricted cell division is the occurence of signs of uncontrolled cell proliferation which has been found to occur in developing chicks deprived of Vitamin E.

It is also suggested that Vitamin E plays a part in the oxidation-reduction processes of the cells, especially in those of the fats. There is little evidence for this as the oxidation processes and general metabolism of Vitamin E deficient animals are not lowered. It is possible that the Vitamin has an anti-oxigenic action, protecting the cells against harmful antoxidising fatty acids, although this is improbable also, as its protecting power does not correspond to its Vitamin E activity in the various types of tocopherol.

The next section deals with the pathological conditions of cells deprived of Vitamin E. In chick embryos it is found that lack of Vitamin E results in some of the cells proliferating in an uncontrolled fashion. Actual lymphosarcoma and reticular cell sarcoma were noted in some of the animals although it is doubtful if these were primarily due to Vitamin E deficiency. The occurence of fibroma of the uterus is apparently more frequent in Vitamin E. deficient rats and some Authors have found that Vitamin E. raised the threshold of resistance to the production of experimental tumours in these animals. Many other Authors have failed to confirm this latter finding. Other Experimentors produced tumours by administration of wheat germ oil, but this was almost certainly due to carcinogenic substances in the oil and not to Vitamin E. However the fact that Vitamin E. is supposed to stimulate the growth of cells, and its deficiency to prevent cellular division, and that it is chemically related to human carcinogenic agents makes it more likely that excess of the vitamin and not a lack should result in malignant changes. This question cannot be cleared up till further experiments are carried out and till it is known if Vitamin E has similar functions in different species of animals.

It was thought that lack of the vitamin might effect the haematological organs. A leukaemic like condition and a haemolytic anaemia have been noted in chicks deprived of Vitamin E. but it has not been proved if this was the real cause of these conditions. The bulk of experimental evidence indicates that Vitamin E excess or deficiency has no effect on the blood picture or on iron metabolism. Vitamin E has no place in the therapy of blood diseases in clinical medicine. It has been tried in wounds and such conditions where cellular proliferation is taking place, but, although there have been some reports of its value in these conditions, they have been insufficient for any conclusions to be drawn from them.

Then the effect of Vitamin E. deficiency on the female reproductive system is considered. There is

no doubt that the vitamin is necessary for the normal completion of pregnancy in rats. If it is deficient the embryo dies and is absorbed. The pathological changes occuring in the embryo prior to and after death are described in detail. It is noted that the foetal placenta is primarily affected, showing such changes as atrophic allantoic projections, but the maternal placenta is apparently only involved secondarily to the foetal changes. Lesions in the embryos of Vitamin E. deficient chicks are also described as they differ from those described in the case of the rat.

In the female rat it is found that after prolonged Vitamin E. deficiency changes occur in their uteri and other reproductive organs which means that Vitamin E. deficiency in these animals is not a truly reversible process as it was once thought to be. These changes are described and it is noted that after each resorption a higher dose of Vitamin E. than before is needed to prevent the next one.

In states of Vitamin E. deficiency in these animals it has been found that if the young are born and live, they usually fail to develop normally if they still have to depend on their mothers for their supply of the vitamin. If the mother manages to produce several litters owing to an incomplete deprivation of the vitamin each litter is nearly always smaller than the last, and their gestations are prolonged owing to death and resorption of some of the embryos in utero. Vitamin E. is apparently not needed for the actual flow of milk in these animals but the offspring are dependent on the supply of the vitamin in the milk as there own stores are inadequate to permit of normal development until weaning.

Next the changes in the pituitary of Vitamin E deficient rats are described. It is noted that both the acidophil and basophil cells may degenerate and the hormone content of the gland may be altered. These findings may have a connection with the disturbance of reproduction, but it seems more probable that they are secondary to them. The thyroids show signs of decreased activity due to the pituitary changes or to a primary effect of the vitamin lack and the adrenal glands are also abnormal.

Then the results of some of Shute's experiments are recorded. He found that in Vitamin E deficient animals the serum contained an oestrogenic like factor which had an antiproteolytic action. This factor resisted the invasion of the trophoblast of the foetal placenta and so death of the embryo resulted with subsequent resorption or abortion.

Some experimentors noted that partial Vitamin E deficiency was more dangerous to rats than complete.

This was apparently due to the kidney damage and toxic symptoms arising from this, as a result of the resorption of a large amount of foetal material which takes place if the death of the foetus does not occur till late in pregnancy.

All these experimental findings prove without a doubt that Vitamin E is needed by certain female animals for the normal functioning of their reproductive systems. It is not nearly so certain that it is required by human beings, and the next section is devoted to a discussion of this problem. The condition in women which most closely corresponds to the changes in experimental animals is Habitual Abortion. Many Authors have recorded what they consider to be the successful treatment of this condition with Vitamin E but it is so difficult to tell how many of the cases would have proceeded normally to full term without any treatment. Malpas's experiments to estimate this latter figure are recorded and it is very little below the number claimed to be cured by the vitamin. The same problems affect the treatment of threatened Abortion and they cannot be cleared up till it is definitely proved whether Vitamin E. plays any definite part in the functions of the human female reproductive systen, and even if it does play a part, it must be proved that it cannot be synthesised in the human body. The theoretical basis for treating these two conditions with Vitamin E. is sufficient to warrant further investigations. These should be done on patients as nearly as possible under the same conditions as regard such matters as diet and environment; and control cases, who do not receive treatment with Vitamin E, should always be included in the experiments. In the present state of our knowledge it cannot be said if Vitamin E is really of any value in the treatment of these conditions but likewise it cannot be said that it is of no value.

Vitamin E. has no effect on female sterility nor from experimental data would it be expected to. However, it may play a rôle in the production of toxaemias of pregnancy and in cases of abruptio placentae. This problem is fully discussed and it need not be repeated here except to note that Shute also found his antiproteolytic factor in the serum of women suffering from these conditions and so concluded that they were also suffering from Vitamin E deficiency. If his theories on the effects of the lack of Vitamin E. are true, it is a good explanation of why Vitamin E. deficiency should result in these conditions. Obviously if the trophoblast is prevented from gaining a firm hold on the uterine wall, parts of the placenta may die and be absorbed, giving rise to toxic symptoms and ultimately the placenta may separate from the uterine wall altogether giving rise to

abruptio placentae.

Most of Shute's experiments were done on women and not on experimental animals, so they are given in detail in this section. He found that women suffering from diseases which were analagous to those found in Vitamin E deficient animals, exhibited an antiproteolytic factor in their serum which was closely allied to oestrin. In vitro serum containing this factor resisted the digestive action of trypsin on its own proteins for a longer time than normal sera did. It disappeared when Vitamin E. was given to the patient and she recovered from the disease. The treatment of various other diseases with Vitamin E. is discussed but there is not enough evidence, positive or negative, in these cases to warrant their mention here.

The theories of the mode of production of these effects are next discussed. It is mentioned that an interference with cellular function is a sound theory. Some Authors consider that the pituitary function is disturbed by lack of Vitamin E. and cite various facts such as histological changes in support of this. Others state that these changes are secondary to the effects of the Vitamin lack on the reproductive organs themselves and this seems the most probable explanation. It is mentioned that disturbed thyroid function may also be primarily due to the lack of Vitamin E, or be secondary to pituitary changes. It is suggested that Vitamin E is necessary for the normal functioning of the sex hormones, but experimental evidence is not in favour of this. Lack of the vitamin may secondarily effect these substances. Finally Shute's theory of an antiproteolytic factor in the serum during Vitamin E deficiency is fully discussed and it is pointed out that although this theory is a good one and fits nearly all the facts, it does not rest on a very sound basis and until further experiments are carried out which will clarify its position, it cannot be accepted as more than a feasable explanation.

The next section is devoted entirely to reports of various Authors on the use of Vitamin E in pathological conditions of the female reproductive system. Habitual abortion is dealt with first and it is found that the average cure rate of all the various series of cases is sixty five per cent, which is not sufficiently positive to prove the value of Vitamin E. one way or the other. The cure rate in cases of threatened abortion is eightyone per cent, but unfortunately most of these cases received other forms of treatment besides Vitamin E, which likewise makes this figure of no particular value. Successes are reported in the treatment of other conditions such as abruptio placentae, defective lactation and senile vaginitis, but these are not of sufficient number to be of any significance. It is noted that congenital abnormalities may be caused by Vitamin E. deficiency of the mother.

The effects of Vitamin E. deficiency on the male reproductive system are now described. The changes found in the testes of rats deprived of Vitamin E. are given in detail. It is noted that signs of degeneration and alterations in the hormone content are found in their pituitaries. The accessory sex glands seem to be but slightly affected. In a few cases somewhat similar testicular changes have been found in human testes, but it is not known if they are due to lack of Vitamin E.

It seems most likely that these changes are due to some fundamental effect such as a disturbance of cellular division. Experimental evidence is against them being secondary to the pituitary changes as these resemble, more than anything else, those seen after castration. Vitamin E. deficiency does not seem to effect the interstitial tissue of the testes which makes it extremely unlikely that the changes are due to disturbances in testicular hormone function. Shute states, in contradiction to other investigators, that the changes are not incompatible with prolonged excess of oestrin in the blood serum.

Vitamin E. has been tried in the treatment of sterility in males, but one would not expect it to be successful as it is found in experimental animals that once the pathological lesions have begun to occur they are irreversible. It has also been tried in other conditions such as undescended testicle.

The next sections are devoted to the nervous and muscular systems. It seems evident that the lesions found in the neuromuscular systems in Vitamin E, deficient animals are due to lack of the same factor that produces the lesions described in the reproductive systems of these animals. It is also unlikely that two factors are needed to prevent the former changes. The many reports of primary muscular dystrophy occuring in various animals such as the rat and the rabbit when deprived of Vitamin E. are described first, although whether these are really primary and not secondary to degeneration of some part of the nervous system, such as the sympathetic centres in the cord, is not certain. The disturbances of the creatine and creatinine metabolism of these animals is noted and the curative effect of Vitamin E on this metabolism described. Then a very detailed description is given of the changes found in the central nervous system of rats deprived of Vitamin E. This has not been described by many investigators and it is possible they do not occur in every species of animal deprived of Vitamin E. They may only occur if the deficiency takes place at a certain age, or if the parts have been previously damaged by another noxious agent. The pathological picture is described as being a cross between Tabes Dorsalis and Amyotrophic Lateral Sclerosis as seen in man. Slight changes are found to occur in the cerebrum of Vitamin E deficient rats, but they are more marked in the brains

of Vitamin E. deficient chicks. Growth and weight in animals is also apparently affected by lack of Vitamin E.

As with the reproductive system there is no doubt that these changes in animals are produced by lack of Vitamin E. but whether diseases in man, which show pathological pictures somewhat analagous to these, are also due to lack of Vitamin E, is very uncertain. To begin with the results of treating nervous diseases with Vitamin E. were very good, but these results have not been confirmed by more recent investigators, and it is possible that these results could be explained by the notoriously remittant nature of chronic nervous diseases or by the psychological effect of any therapy given to these patients. The pathological changes found in muscular dystrophies and chronic nervous diseases in man are not exactly similar to like conditions found in Vitamin E deficient animals and, just because they do bear some resemblance, it does not prove they are due to lack of the vitamin. The question whether Muscular Dystrophies in man might be due to Vitamin E deficiency is discussed first and then whether motor neurone disease might be due to it. This latter disease is the one whose pathological picture corresponds most closely with the changes found in the central nervous system of Vitamin E deficient animals. The various reasons why these diseases have been thought to be due to Vitamin E. deficiency nedd not be repeated as none of them are in any way conclusive. The juvenile dystrophies are probably the diseases most akin to the muscular dystrophies of Vitamin E. deficient animals. It may be noted that although successful reports of the treatment of these diseases have been recorded one would not expect this except possibly in early cases, as most experiments on Vitamin E deficient animals show that the vitamin is of great value in the prophylaxis of the neuromuscular changes, but of little value in their treatment. Once the changes have begun to occur, they appear in most cases to be irreversible unless prevented in their very early stages. The disturbed Creatine and Cratinine metabolism of these diseases which is definitely affected by Vitamin E in animals, appears to be unaffected in man which suggest that these conditions in man and in animals are not strictly analagous.

Vitamin E. has been used in the treatment of many other nervous diseases such as Tabes Dorsalis Cerebral Palsies and Polyneuritis, but the results have been disappointing and the reports are not of sufficient number to warrant any conclusions being drawn from them. The theoretical basis for the treatment of most of these diseases with Vitamin E. is slight in the extreme.

The next section is devoted to the theories suggested to explain these effects of Vitamin E deficiency. Again a disturbance of cytological metabolism seems to be a distinct possibility. It is difficult to see how disturbances of Pituitary function could give rise to these changes and if

this is the case it is unlikely that they cause the reproductive changes either as there should be a common cause for all the effects of Vitamin E deficiency. A link has been found between cases of muscular dystrophy and disturbances of the reproductive system and it has been noted that insufficiency in proteolytic enzymes in the blood results in muscular dystrophies in some animals. This suggests that Shute's theory of a balance between Vitamin E and an antiproteolytic factor might explain these neuro-muscular changes as well as the reproductive ones, although it does not account for the lesions of the central nervous system. Finally it has been suggested that Vitamin E may protect the central nervous system against the invasion of certain viruses.

The last section deals with the results of treating patients suffering from neuromuscular diseases with Vitamin E. Only twentytwo per cent of the cases of muscular dystrophy and nineteen per cent of the cases of motor neurone disease treated with Vitamin E by various Authors were reported to show definite improvement as a result of this treatment, but this is as would be expected even if the diseases were definitely known to be due to lack of Vitamin E, because, as has already been noted, once the changes begin to occur they soon become irreversible. The fact that many of the cases were treated by other substances as well as Vitamin E makes these figures even less conclusive than they are already. In future investigations this multiple therapy should be avoided if possible. Many other diseases have been treated with Vitamin E such as Tabes Dorsalis, Neuritis and Fibrositis, but there is no real basis for this and the reports have not been sufficiently numerous to enable conclusions to be drawn one way or another as to whether the treatment is of real value or not.

In conclusion it may be stated that the effects of Vitamin E. deficiency are most probably caused by a fundamental disturbance of cellular function which manifests itself acutely in certain rapidly dividing cells. The next most likely explanation is the theory of a balance existing between Vitamin E and an antiproteolytic factor in the blood serum. It cannot be stated definitely if diseases in human beings are caused by Vitamin E deficiency. It is justifiable to carry out further investigation on the treatment of cases of habitual and threatened abortion and possibly of abruptio placentae with Vitamin E, as, if these conditions are due to deficiency or Vitamin E, they would be expected to respond to treatment. Owing to the irreversible nature of the neuromuscular changes good results in the treatment of diseases such as muscular dystrophy and motor neurone degeneration can never be expected even if they are proved to be definitely due to Vitamin E deficiency. The value of Vitamin E in these neuromuscular diseases is undoubtedly in their prophylaxis, but it is impossible to see how

practical use can be made of this except perhaps in infants who are born of mothers known to be taking a poor diet. It seems that the position of Vitamin E in the causation and treatment of diseases in man will only be cleared up when tests can be simply carried out to estimate the amounts of Vitamin E present in the body fluids and tissues, and it is found whether these amounts vary in states of disease and under the effect of treatment with the vitamin. From these findings it should be possible to decide whether the Vitamin is really necessary for the functions of the human body. A start in this has been made in the case of patients, suffering from Amyotrophic Lateral Sclerosis, and treated with Vitamin E. It was found by Emmerie and Engel's method that the quantities in their serum were not less than in normal cases and that treatment with the vitamin raised these quantities, although only a few of the cases improved clinically. Fitting this in with the two most likely theories mentioned above, this seems to suggest that the disease is produced, not by a lack of the vitamin, but either by an inability of cells to utilise the vitamin due to some unexplained and unknown factor, or by an excess of the oestrogenic antiproteolytic factor in the serum, produced by some equally unknown process, which needs abnormally large quantities of the vitamin to neutralise it. Admittedly this is pure hypothesis but it does seem to indicate the trend that investigations should take in the future if anything more definite is to be proved on this subject. The uncontrolled treatment of miscellaneous diseases with the vitamin is never likely to lead to definite conclusions as none of them, which are at all likely to be due to the deficiency, have a sufficiently well defined and regular course to enable the result of their treatment to be interpreted in a cut and dried manner. Probably the first step is to find a simpler test, than that devised by Emmerie and Engle, for the estimation of the amounts of Vitamin E present in the various tissues and fluids of the body.

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